THE 1988 FENITROTHION MONITORING STUDY ON SONGBIRD BRAIN CHOLINESTERASE ACTIVITY

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EXECUTIVE SUMMARY

Background Information

The Newfoundland and Labrador Department of Forestry conducted an aerial spray program to control an outbreak of the eastern hemlock looper (Lambdina fiscellaria fiscellaria) in insular Newfoundland during the summer of 1988. The primary insecticide used in the spray application was fenitrothion.

The Department of Forestry (DF) was required as a condition of their Operator's Licence to undertake a monitoring study to assess the effect of fenitrothion on forest songbirds.

This monitoring study was designed to describe the effect of two operational fenitrothion applications on the ChE activity of songbirds. This is not a monitoring study investigating mortality, the fate of affected songbirds or the behavioural responses of songbirds following two operational spray applications.

The monitoring study area comprised five spray blocks located between Daniel's Harbour and the Port Saunders area of the Northern Peninsula.

The methodology chosen for the monitoring study used White-throated Sparrows (WTS) brain cholinesterase (ChE) activity as the indicator of spray effect. WTS are abundant, easily collected and have been used as monitoring species in other studies. WTS were collected using .410 shotguns. A control sample of WTS was collected prior to any spray activity to determine normal brain ChE activity in WTS. WTS brains were removed and stored in liquid nitrogen for later ChE determination. Only whole brains from adult male WTS were used in the analysis.

It was necessary to establish criteria by which ChE activity could be judged normal or depressed. The values calculated by Ludke et al. (1975) and Zinkl et al. (1980) were used in the data analysis to determine whether or not any particular songbird collected after spraying had a normal or depressed ChE activity. The values used were the WTS control sample mean less two standard deviations and the mean less 20% of the mean. "Normal" birds would not likely have ChE activity below these values. If a WTS was collected from a sprayed area and its ChE activity was below the lower of these two values it was considered depressed due to the fenitrothion spray. Since these calculations give different values, the one giving the lowest value was used when determining whether an activity was depressed.

Field Results

In general, WTS brain ChE activity was depressed the maximum amount on the Day 1 after the first spray. An apparent rapid recovery in ChE activity was observed on the Day 3 after the first spray. The ChE activity in WTS following the second spray application did not produce the same response curves as the ChE activity monitored after the first spray application. The exact reason for the difference in response curves is not known and could not be determined from this study. A possible explanation was provided based on the biological consequences of ChE depression in songbirds.

The overall effect on songbirds of the operational spray delivery in 1988 was less compared to the 1987 and 1986 spray programs. However, in 1988 severe abnormal behaviour in songbirds was observed by field personnel for the first time. Approximately 58% of all the WTS collected in this study had ChE activity indicating exposure to fenitrothion, and approximately 9% had ChE activity indicating severe or potentially life-threatening exposure.

The songbird collecting method used in this study is strongly suspected of underestimating the extent of severely affected songbirds being sampled. This is because songbirds with severe ChE depression are not likely to be collected by the field crews. With this sampling bias taken into consideration, 9% of the songbirds collected exhibit a potentially life-threatening depression in brain ChE must be considered significant.

Results of the second spray effects lend support to the argument proposed that the apparent "rapid recovery" of exposed songbirds on Day 3 following the first spray is more likely due to the sampling bias than a true recovery.

Three incidents of abnormal songbird behaviour after a fenitrothion spray delivery were reported by the field crews in 1988.

Conclusions

The results of the 1988 monitoring study indicate that the safety of forest songbirds during operational fenitrothion spray activities is a concern. Concern is not only based on the potential for direct songbird mortality in the field, but the adverse biological consequences of sublethal ChE depression on the breeding performance of Newfoundland songbirds.

The typical sharp first spray related depression in ChE activity followed by a rapid increase in ChE activity as indicating recovery from fenitrothion exposure is questioned. Based on the biological consequences of ChE depression, the rapid "recovery" is suggested to reflect the sampling bias in the methodology. The impact of operationally applied fenitrothion on songbirds is considered to

be underestimated using the shotgun method of sampling.

Recommendations

Future fenitrothion monitoring efforts should be designed to address biological concerns over the fate of severely affected individual songbirds and the effect of sublethal ChE depression on reproductive performance (i.e. population level effects monitoring is needed).

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1.0 INTRODUCTION

The Newfoundland and Labrador Department of Forestry (DF) has used fenitrothion, an organophosphorus pesticide, from 1985 - 1988 to control infestations of the eastern hemlock looper (Lambdina f. fiscellaria) in insular Newfoundland. Fenitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate) is one of many organophosphorus (OP) pesticides used to control lepidopterous and other insect pests in North America.

Throughout their aerial spray program, DF has annually monitored the effects of the spray application on songbirds as a condition of their Operator's License. OP pesticides act to inhibit cholinesterase (ChE) (Appendix A). Analysis of ChE activity has been the method used to monitor the effect of fenitrothion on songbirds.

Since ChE activity is easily measured, its measurement can be used to determine if a bird has been exposed to OP pesticides (Stickel 1974). ChE determination is becoming widely accepted as a method of assessing exposure of birds to ChE inhibiting pesticides (e.g. White et al. 1979; Zinkl et al. 1979, 1981; Busby et al. 1981, 1983; Strong and Wells 1986; Mineau and Peakall 1984, 1987; Lemon 1987, 1988; Hill 1988).

Strong and Wells (1986) reported that between 29-100% of all individual songbirds collected from spray blocks had ChE activity less than 80% of average values for those species collected from control areas indicating probable exposure to fenitrothion during the 1985 DF fenitrothion monitoring study.

In 1986, one operational spray block was monitored for DF in which ChE activity levels were found to be reduced in all songbird species sampled and in 38% of the songbirds collected the ChE depressions were $\geq$50% (Lemon

1987). Based on the 1986 results the monitoring program was expanded to include six operational spray blocks in 1987 (Lemon 1988). The 1987 monitoring study provided an opportunity to assess variability amongst spray blocks in the observed effect of the operationally applied fenitrothion on songbirds as determined by ChE activity.

In 1987, 50% of the songbirds collected had depressed ChE activity and 17% of the songbirds had $\geq$50% ChE depression compared to control samples following exposure to fenitrothion (Lemon 1988). However, 90% of the songbirds collected with ChE activity depressed $\geq$50% from control samples were collected from two of the six spray blocks indicating variability in the exposure of songbirds to fenitrothion amongst spray blocks (Lemon 1988).

One of the 1987 monitoring blocks involved collecting songbirds following both fenitrothion spray applications. There was evidence of ChE recovery toward pre-spray levels after four days of ChE monitoring following the first application, however ChE activity continued to decline throughout the four day monitoring period following the second application (Lemon 1988).

On the basis of previous results, in 1988 it was decided to monitor songbird ChE activity in four operational spray blocks and conduct a more thorough investigation of second spray effects on songbird ChE activity.

S. Fudge and Associates Limited was contracted by DF to conduct the songbird monitoring study for the 1988 spray program in western Newfoundland according to the Terms of Reference (Appendix B). A review of the operational use and effects of fenitrothion and other organophosphorus insecticides used to control outbreaks of lepidopterous insect pests is presented in Zinkl et al. (1980), Peakall and Bart (1983), and Mineau and Peakall (1987) and Lemon (1988).

1.1 ORGANOPHOSPHORUS PESTICIDES AND ChE DEPRESSION

A brief summary of a review of organophosphorus (OP) pesticides and ChE depression in altricial songbirds is provided in this Section. A more detailed review of the pertinent literature is provided in Appendix A.

Fenitrothion, as well as other OP pesticides, inhibits ChE activity, which is an enzyme required in the "normal" functioning of songbirds or any vertebrate. Following exposure to fenitrothion, songbirds may exhibit symptoms ranging from no real noticeable behaviourial effect in the case of minor exposure to an effect that is seriously debilitating to the "normal" functioning of a wild bird. The amount of ChE inhibition observed in songbirds appears directly related to the amount of fenitrothion the bird ingests or is exposed to. In this study only the application rate of fenitrothion is known. Because the exposure to fenitrothion or the amount of fenitrothion ingested by the birds is not being measured, songbirds with greater ChE inhibition are assumed to have been exposed to or have ingested more fenitrothion than songbirds with a lesser degree of ChE inhibition.

One obvious bias (see Section 2.4) inherent in the shotgun collecting method employed in this study to obtain brain samples is that the method would select for less affected songbirds (Mineau and Peakall 1987). More "normally" behaving songbirds that are less affected (less exposure) by the OP would be more easily collected by fieldworkers. Although attempts were made to minimize this collecting bias, it should be a factor considered when interpreting the results.

The biological consequences of ChE depression as determined from a brief review (see Appendix A for details) of the literature on laboratory and field research follows:

i) exposure to OP pesticides, including fenitrothion, can result in songbird mortality

ii) sublethal ChE depression is reversible

iii) sublethal ChE depression in adult birds produced a trend toward reduced reproductive performance in the laboratory

iv) sublethal ChE depression in Starling nestlings (exposed at day 16) did not adversely affect nestling mass at fledging or postfledging survival

v) nestlings of altricial songbirds have lower concentrations of ChE than adults and are exposed to OP pesticides primarily through ingestion of contaminated food from adults in addition to inhalation and possibly through contact with the plumage of attending adults

vi) research indicates that sublethal ChE depression in nestling atricial songbirds may result in reduced weight gain and reduced adult reproductive success.

1.2 OBJECTIVES

The objective of this study was to monitor the effects of two operational applications of fenitrothion on the ChE activity of White-throated Sparrows (Zonotrichia albicollis) during the 1988 DF program to control the outbreak of eastern hemlock looper in Newfoundland.

1.3 STUDY AREA

Monitoring efforts were concentrated in four operational spray blocks situated between Daniel's Harbour and the Port Saunders area of the Northern

Peninsula. The spray blocks were located in the Northern Peninsula Ecoregion which is characterized by balsam fir (Abies balsamea) and black spruce (Picea mariana) forests (Damman 1983). The spray blocks were situated on the largely bog-covered coastal plain where the lack of forest fires have resulted in the predominantly balsam fir forest cover (Damman 1983).

The location of the monitored spray blocks are shown in Figure 1.1.

1.4 MATERIALS AND METHODS

1.4.1 Spray Application Details

The technical details of the spray application and spray mixture are provided in Tables 1.1 and 1.2.

1.4.2 Sampling Design

White-throated Sparrows (WTS) were collected prior to scheduled spray operations to provide researchers with a control sample with which to compare WTS collected following fenitrothion applications (Appendix B). The collection schedule for WTS following spray delivery is presented in Table 1.2.

The post-spray collection schedule was designed to document the recovery of ChE activity in WTS following a predicted prolonged depression resulting from exposure to two fenitrothion applications of 210 g AI/ha (Lemon 1988).

Four spray blocks were originally scheduled to be monitored for the 1988 project. However, time constraints precluded the four blocks from receiving complete two-spray monitoring efforts. Instead, three spray blocks received

Figure 1.1

LOCATION OF MONITORING

STUDY SPRAY BLOCKS

1988

SCALE 1:500 000

10 0 10 20 30 km

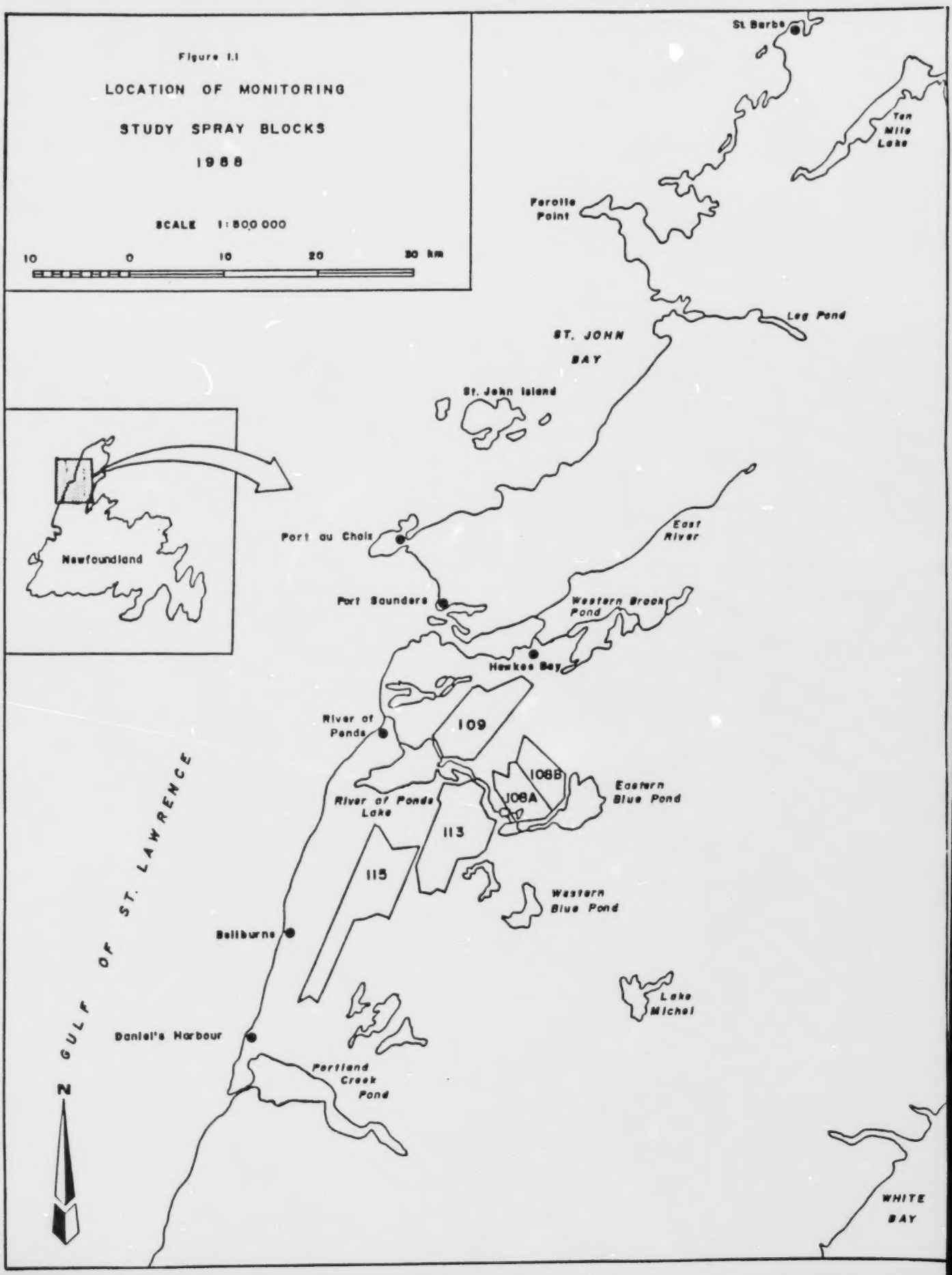

Table 1.1 Technical details of spray application and formulation for the 1988 hemlock looper spray monitoring project.

Spray Application

Aircraft: Ayres Bull Thrush - 4 aircraft working in a team

Spray Height: 15 m above canopy

Spray Speed: 217 km per hr.

Spray Apparatus: Mini-Micronair - AU 5000 - 6 per aircraft

Swath Width: 122 m per aircraft

Spray Mixture

Insecticide: Fenitrothion (Folithion ^{R})

Mixture by volume:
11% fenitrothion
40% Cyclosol 63 (solvent)
49% ID 585 (insecticide diluent)

Application Rate: 2 applications of 210 g active ingredient per 1.5 L per ha per application

Table 1.2 Spray dates and White-throated Sparrow (WTS) sampling schedule for the 1988 hemlock looper spray monitoring project.

Block	Spray Date	Start Finish	WTS Collections Day 0	Day 1	Day 3	Day 5	Day 7
108A							
1st spray	88-07-07	0805-0825h	88-07-07	88-07-08	88-07-10		
2nd spray	88-07-23	2142-2216h	88-07-24	88-07-25	88-07-27		
108B							
1st spray	88-07-12	2042-2116h	88-07-13	88-07-14	88-07-16		
2nd spray	Not Used in Monitoring Study						
109							
1st spray	88-07-05	0643-0716h	88-07-05	88-07-06	88-07-08		
2nd spray	88-07-18	2122-2155h	88-07-19	88-07-20	88-07-22	88-07-24	88-07-26
113							
1st spray	88-07-14	0800-0854h	88-07-15	88-07-16	88-07-18		
2nd spray	88-07-20	0834-0929h	88-07-21	88-07-22	88-07-24	88-07-26	88-07-28
115							
1st spray	88-07-07	0615-0656h	88-07-07	88-07-08	88-07-10		
2nd spray	88-07-18	0640-0819h	88-07-18	88-07-19	88-07-21	88-07-23	88-07-25

the complete two-spray monitoring efforts. One other block received monitoring efforts after the first spray and three days after the second spray, while two other blocks received monitoring efforts after the first spray only (see Table 1.2).

1.4.3 Songbird Collections

Adult male WTS were collected by shooting using .410 calibre shotguns with #6, #7 1/2 or #9 lead shot. Collections began no sooner than 2 h after the spray had been completed to avoid field researchers from coming into contact with the spray in aerosol form. WTS were usually located by song, then "pished in" or "whistled in" to obtain a clear shot at close range. Hunting was avoided in areas near the spray boundaries and designated shut-off areas within the blocks to reduce the chance of collecting unexposed WTS that flew into the sprayed area after spraying.

If a block was sprayed in the evening, the collectors were in the field the next morning at daybreak trying to collect WTS. Due to the relatively small size of the spray blocks and limited vehicle access, collection day sample sizes were reduced from 10 to approximately seven. In addition, efforts were made on each collection day to collect WTS from different areas in each block to avoid removing too many WTS from any particular location.

After collection, WTS were taken to an area where a liquid nitrogen container was located. Brains were removed from the WTS, placed in serum vials and stored in liquid nitrogen until time of assay. Generally, specimens were processed within 1 h of collecting and never longer than 2 h. For each WTS, the percentage of brain that remained was recorded after collection was recorded. Brains which had received heavy shot damage were not used in the analysis because different areas of the brain are reported to have different levels ChE of activity. Statistical analysis was conducted on data

from whole (>95% total brain) brains collected from adult male WTS. This was necessary to avoid any possible differences in ChE activity due to sex or age (immature vs. mature) or varying ChE concentrations in different portions of the brain.

ChE assays were conducted by the Canadian Wildlife Service, Fredericton, New Brunswick and followed the colorimetric techniques of Ellman et al. (1961) as adapted by Hill and Fleming (1982).

Whole brain samples were used in the laboratory analysis. Samples were stored in coded vials such that CWS laboratory personnel had no way of knowing the history of any particular specimen. The final reported ChE value was an average of 2 assays on each brain sample. Prior to assaying, all samples were randomized to prevent possible bias from processing sequential groups of specimens under laboratory conditions which can vary slightly from day to day. Brain tissue analysis was completed on 13 September 1988. Individual WTS ChE activity and the exact locations of each WTS collected appears in Appendix C.

Field researchers recorded songbird activity and any behaviourial abnormalities in songbirds observed in the field following spray application.

1.4.4 Data Analysis

Birds collected in each block on the different collection days were considered to be individual samples. The data on the level of brain ChE activity from the chemical analysis were sorted according to day of collection within each block. These data were tested for normality and homogeneity of variance. Differences among blocks and days of collection were analyzed using non-parametric tests if the data was skewed or heterogeneous, otherwise parametric tests were used. The level of rejection for all statistical tests was P=0.05.

These data were also viewed according to the criteria of Zinkl et al. (1980). The standard, threshold ChE activity below which songbirds are considered to have been exposed to a ChE inhibiting agent is the lower of two values, either the control sample mean ($\overline{X}$) -.2$\overline{X}$ or $\overline{X}$-2 SD (standard deviation) (Zinkl et al. 1980). A $\geq$50% inhibition of brain cholinesterase ($\overline{X}$ -.5$\overline{X}$) is considered to be potentially life-threatening (Zinkl et al. 1980, Ludke et al. 1975). Because of the acknowledged sampling bias that is expected to result from collecting mainly "healthy" birds and to facilitate inter-study comparisons (see Appendix A, and Mineau and Peakall 1987), the data were also presented as proportions of individuals in the sample that exhibit ChE depression and $\geq$50% ChE reduction.

2.0 RESULTS AND DISCUSSION

2.1 OBSERVED WHITE-THROATED SPARROW ChE INHIBITION

The ChE response in WTS sample groups following operational fenitrothion spraying for all monitored blocks is shown in Figure 2.1. Details of the collection sample data (sample size, standard deviation and range) are provided in Appendix D.

2.2 STATISTICAL ANALYSIS OF DATA

2.2.1 Calculation of Critical Values

2.2.1.1 Exposure

The critical value below which WTS ChE activity indicates exposure to fenitrothion is the lower of the control sample $\overline{X}$-2 SD or the $\overline{X}$-20% of $\overline{X}$ according to the criteria of Zinkl et al. (1980). The $\overline{X}$ ChE activity of the control WTS samples is 26.0 μmoles/min/g (henceforth referred to as units).

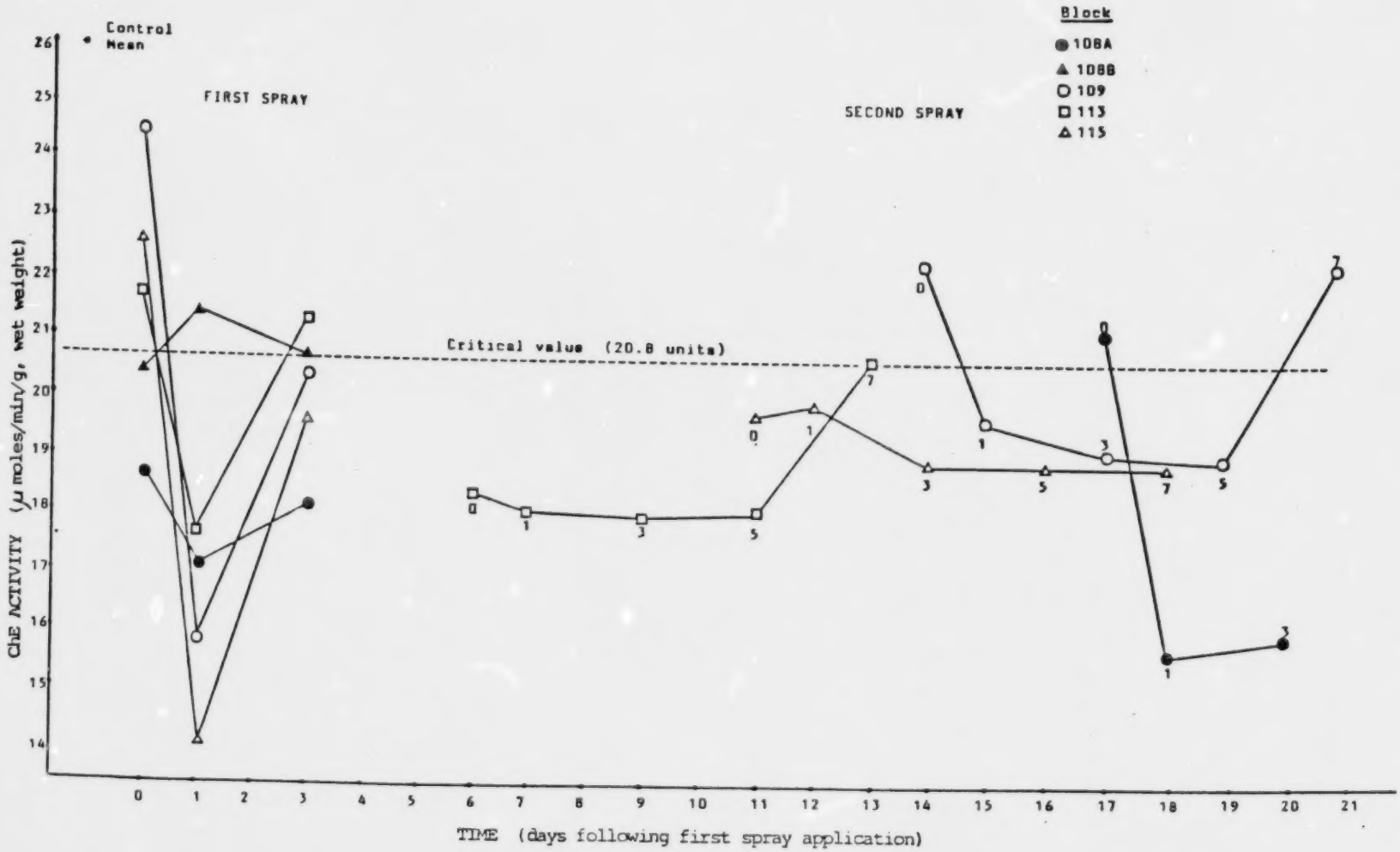

Figure 2.1 Brain ChE activity in White-throated Sparrows collected following two operational applications of fenitrothion (210 g AI/ha).

The sample $\overline{X}$-20%=20.8 units which is a lower value than the $\overline{X}$-2 SD.

2.2.1.2 Life-threatening Exposure

A potentially life-threatening reduction in ChE activity was arbitrarily set at ≥50% of the control sample mean (Zinkl et al. 1980). This does not necessarily mean that a songbird experiencing ≥50% ChE depression is going to die. However, any songbird found dead following exposure to a ChE inhibiting pesticide with a ≥50% ChE depression can be assumed to have likely perished due to ChE depression. Obviously any WTS samples collected in this study exhibiting ≥50% ChE depression were not dead or else they could not have been collected by using the methods of this study.

The use of the critical value and life-threatening ChE exposure facilitates inter-study comparisons. For this study the life-threatening critical value is 13.0 units.

2.2.2 **Statistical Analysis - First Spray**

For the first spray, a 2-way ANOVA test was performed to determine if there was a difference amongst spray blocks and to test if there was a difference among collection spray days. There was no significant differences amongst spray blocks (2-way ANOVA: F=0.90, block d.f.=4, day d.f. =2, P=0.53) so the blocks could then be grouped for further comparison. There was a significant difference observed among collection days (2-way ANOVA: F=7.91, day d.f.=2, block d.f. =4, P=0.00). The result from collection analysis indicate that there are differences between control, Day 0, Day 1 and Day 3 samples.

2.2.2.1 Differences Among Collection Days

The control values are significantly different from Day 0, Day 1 and Day 3; Day 1 is significantly different from control, Day 0 and Day 3; but Day 0 and Day 3 are not significantly different from each other (1-way ANOVA: F=15.71, d.f.=3. P<0.01; Duncan's and Newman-Kuel's multiple range tests).

2.2.3 Statistical Analysis - Second Spray

There is a significant difference amongst the four blocks tested therefore they cannot be combined for comparison (2-way ANOVA: F=2.62, block d.f.=3, day d.f. =4, P=0.05). Results of the multiple range test indicate that Block 108A is different from Block 109 but that Block 109 is not significantly different from Blocks 113 and 115.

If Block 108A is omitted from this analysis (because this block did not receive second spray monitoring effort) then the results indicate that there is no difference between spray blocks and they can be combined for comparison (2-way ANOVA: F=1.68, block d.f.=2, day d.f. =4, P=0.19).

Block 108A was significantly different than the other three blocks. Therefore the subsequent analysis of the data following the second spray is provided for Blocks 109, 113 and 115 combined and as well for Blocks 108A, 109, 113 and 115 combined.

2.2.4 Che Activity Observed Compared to Critical Value

The number and percent of WTS collected with ChE activity below the critical value (20.8 units) and the life-threatening value (13.0 units) are presented in Figures 2.2 and 2.3 respectively. A summary of the results of 1-tailed T-tests comparing the sample means of each collection day to the

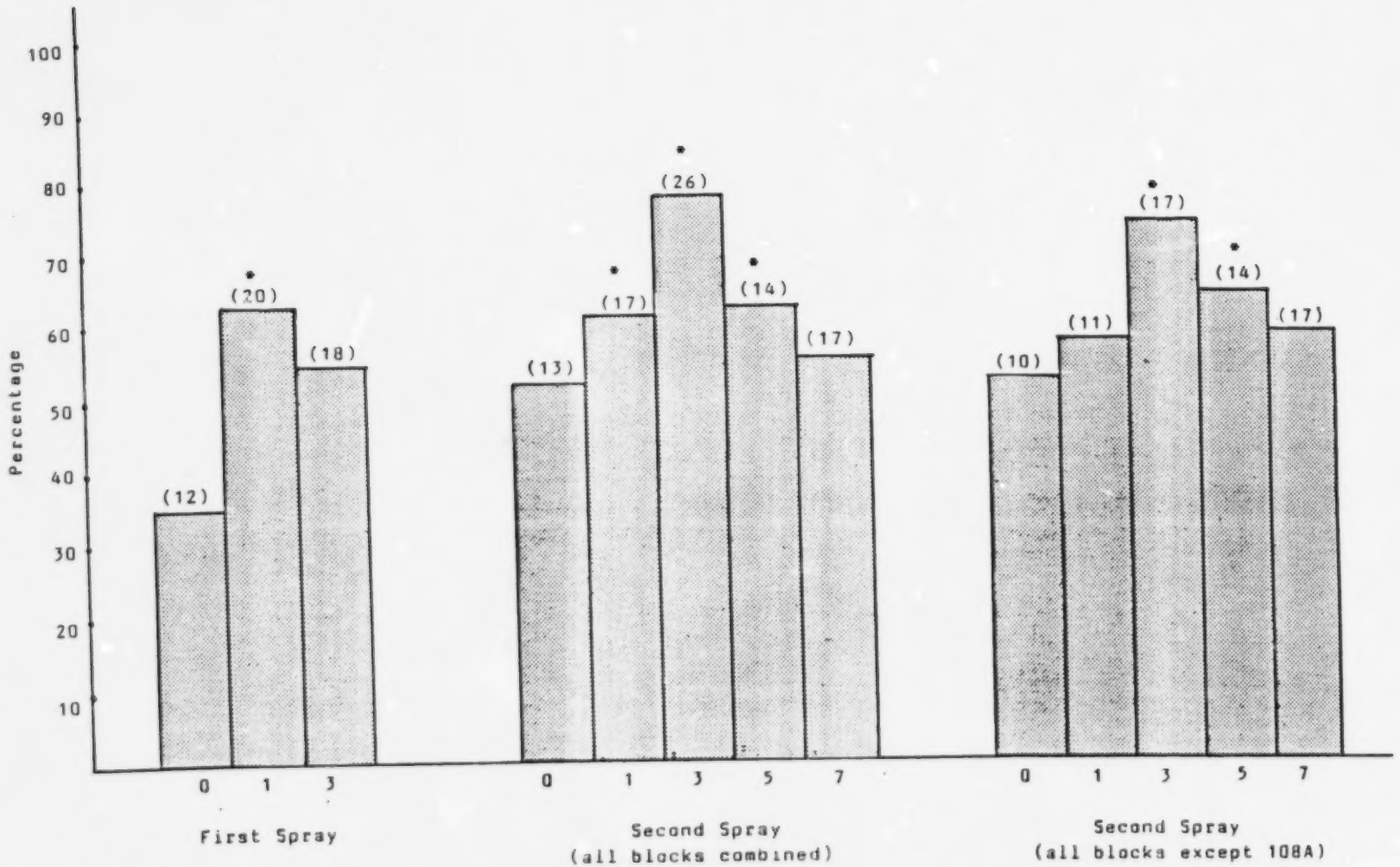

Figure 2.2 Percentage of birds with ChE activity levels below the critical value (20.8 units) on days following spray 1 & 2.

( ) Represents number of samples below critical value.
• ChE activity significantly less than the critical value of 20.8 units (see Appendix D).

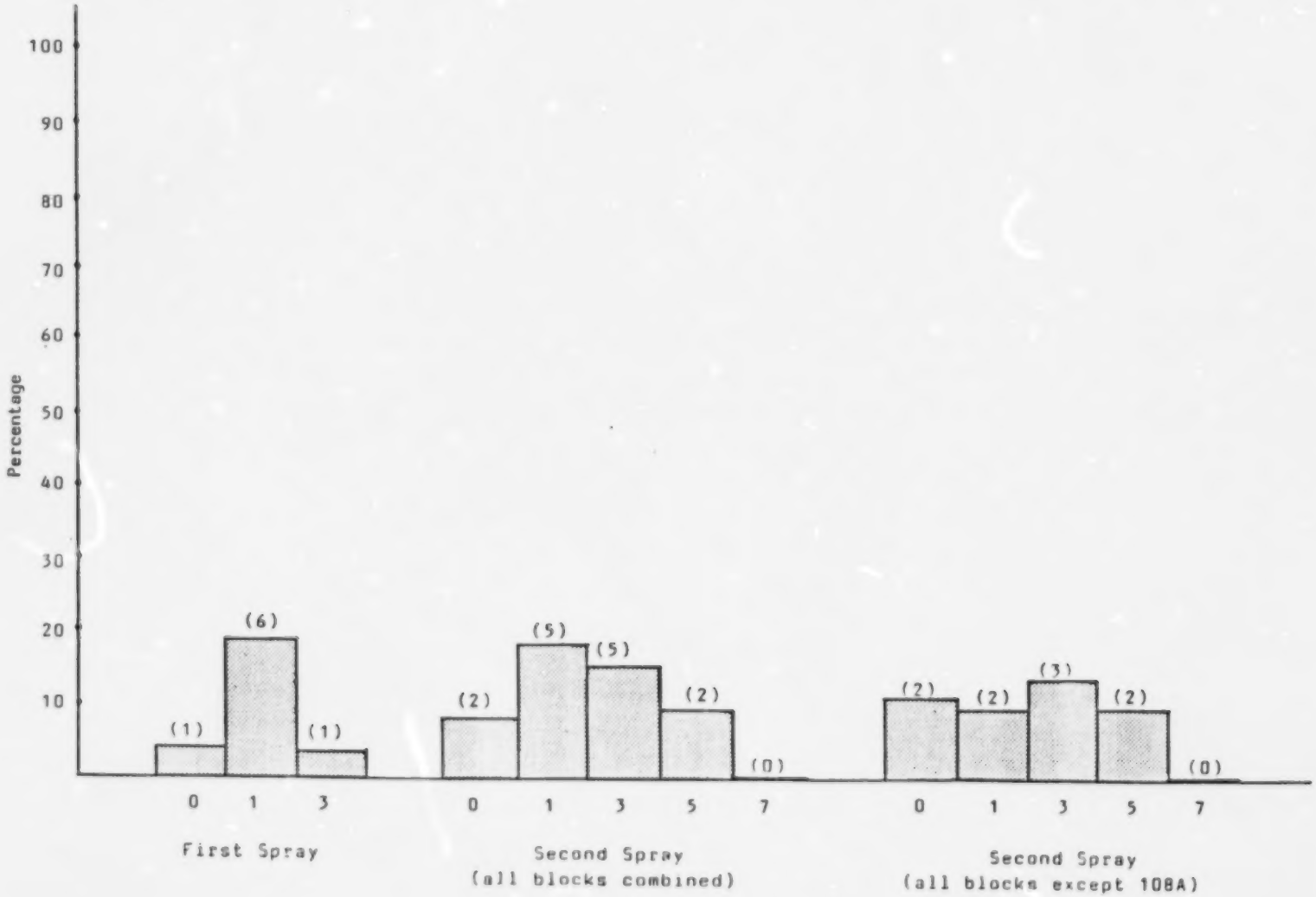

Figure 2.3 Percentage of birds with ChE activity levels below the life-threatening value (13.0 units) on days following spray 1 & 2.

( ) Represents number of samples below 13.0 units.

critical ChE value (20.8 units) is presented in Appendix E.

The total number of all WTS collected that had a ChE activity below the critical value of 20.8 units was 137 or 58.8% of the post-spray samples. This compares with approximately 50% of all samples collected in 1987 and 70% of all samples collected in 1986 (Lemon 1988, 1987).

The total number of all WTS collected that had a ChE activity of 13.0 units or less was 22 or 9.4% of the post-spray samples. This compares to approximately 17% of all samples collected in 1987 and 38% of all samples collected in 1986 (Lemon 1988, 1987).

The severely affected songbirds in 1987 were collected primarily from two spray blocks and in 1986 only one spray block was monitored. Both the 1987 and 1986 spray monitoring studies involved only blocks sprayed by a large aircraft (DC-4) with boom and nozzle spray apparatus.

2.3 DATA TRENDS

2.3.1 First Spray Application

The effect of the first application of fenitrothion produced a consistent response in ChE activity in the WTS sampled (Figure 2.1). Spray Block 108B (an evening spray) had the only peculiar data point where the Day 1 sample mean did not produce the maximum ChE depression observed. However, the Block 108B data was not significantly different from the other blocks following the first spray (2-way ANOVA see Section 2.2.2).

The results are similar to those reported by Lemon (1988). Maximum ChE depression does not occur on Day 0 but on Day 1, and there appears to be a rapid "recovery" of ChE activity by Day 3 after first spray application. This

was also observed during the "extensive" spray blocks monitored in 1987 and reported in Lemon (1988).

The response observed following the first application shows the general dramatic short-term effects that can be expected from fenitrothion on songbird ChE activity (E. Hill, K. Stromborg, pers. comm.). However, the inhibitory effects of fenitrothion appear to be short-lived and ChE depression is usually followed by a rapid trend toward recovery within four days following the first spray application as shown in Figure 2.1 and Lemon (1988). Although the sampling methodology underestimates the ChE depression (Appendix A, and Mineau and Peakall 1987), WTS experiencing only sublethal ChE depression would appear to be recovering within several days following exposure.

Songbirds, in general, receiving sublethal ChE depression from fenitrothion or other OP pesticides, will not fully recover to normal levels for upwards to 30 days (E. Hill, pers. comm.). In this study, the WTS (songbirds) appear to have the ability to recover from sublethal ChE depression following the first of two sprays, since ChE values on Day 3 seemed to be approaching 'normal' levels.

2.3.2 Second Spray Application

The ChE response of WTS exposed to a second fenitrothion application was not similar to the response observed following the first application (Figure 2.1). The second exposure to fenitrothion produced a prolonged depression in ChE activity rather than an apparently rapid recovery following first exposure. A similar trend was reported for the "intensive" spray block in Lemon (1988).

The difference between the first application and second application curves shown in Figure 2.1 may be partially explained by the pesticide detoxification mechanism involved in songbird livers. Organophosphorus (OP) and carbamate pesticide detoxification by vertebrates are both enzyme (catabolic) driven. That means enzymes produced by the affected organisms are used to catabolize (break down) the poison. The difference between an OP pesticide such as fenitrothion and a carbamate pesticide is the action of the enzymes. The rate of recovery of ChE inhibition caused by fenitrothion is governed by de novo enzyme synthesis by the songbird because enzymes binding with fenitrothion do not uncouple (to be used again) from the OP molecule after their action is completed. In contrast, enzymes acting to catabolize carbamate pesticides uncouple from the carbamate molecule after their action is completed and can be used again to bind with other carbamate molecules (E. Hill, pers. comm.).

Therefore, the rate of ChE recovery following fenitrothion induced ChE depression is determined by the rate of new enzyme synthesis. This process of ChE recovery is helpful when trying to rationalize the differences observed between the response curves following the two fenitrothion applications (Figure 2.1). The pattern of ChE recovery should be similar following the two applications, since the songbirds are exposed to the same amount of fenitrothion. The differences in the two groups of curves lends support to the idea that the 'rapid recovery' observed on Day 3 (first spray) is indeed a sampling phenomenon (see Section 1.1, Section 2.4 and Lemon 1988). Songbirds with severe ChE depression may not occur in the sampled population because it is not possible to collect them using the shotgun method of collection. Therefore, it is suggested that severely affected songbirds become unable to be sampled, thus indicating a ChE 'recovery' when actually only less severely affected songbirds are being sampled.

2.4 OBSERVED DIFFERENCES IN THE DATA

The most obvious observed difference in the data presented in this report is in the ChE response curves following the two spray applications. A relatively sharp curve in the ChE depression and recovery is observed following the first spray and a variety of relatively flat curves of depression and recovery follow the second spray.

After several years of monitoring efforts it is apparent that all songbirds in any particular sample are not exposed to the same amount of fenitrothion. This is shown by the range and SD of the mean sample ChE activity (Appendix D). In general, the more fenitrothion any given songbird ingests the greater the ChE depression that follows. The more depressed the ChE activity is the greater the effects on the songbird. Section 1.1 summarizes the effects of ChE depression in songbirds.

Based on Section 1.1 (and Appendix A), a plausible explanation of the observed results (Figure 2.1) following the two spray applications follows. **The reader must keep in mind that this monitoring study was designed to describe the effect of two operational fenitrothion applications on the ChE activity of WTS. This is <u>not</u> a monitoring study investigating mortality the fate of affected songbirds or the behaviourial responses of songbirds following two operational spray applications.**

When comparing the two sprays it is important to realise that they were identical from an application standpoint. In addition to assuming that all spray blocks received the same treatment, for the purposes of this monitoring study and the interpretation of the data, it is assumed that all blocks are similar in terms of prey availability and accessibility. Therefore it is argued that the differences in the two groups of curves following the applications must reflect the phenomenon of the sampling bias. The difference in the

two exposure curves may also reflect differences in the exposure to and/or ingestion of the pesticide by WTS following the two applications.

2.4.1 The Sampling Bias

The sampling bias outlined in Section 1.1 is inherent when using the collection methods described. The sampling favours less affected or more "normally" behaving WTS. WTS that are severely affected by the fenitrothion resulting in them becoming lethargic, non-vocal or inactive would tend to make them difficult to collect.

Some WTS may be predisposed to high pesticide exposure (more active due to territorial behaviour, increased foraging to feed young). If these individuals consequently suffer the most severe adverse effects of the spray, they would tend to be excluded from the sampled population on subsequent days. Therefore, the less affected WTS would tend to make up the following collection day samples. The apparent rapid "recovery" in ChE activity on Day 3 following the first spray is remarkable considering that active fenitrothion probably still persists in the environment and WTS are still exposed during the period of ChE recovery. This rapid "recovery" is not observed in any of the curves following the second application of fenitrothion and, in fact, the rapid decrease in ChE activity is only observed in Blocks 109 and 108A.

The explanation offered in an attempt to explain this difference is that severely affected individual WTS are quiescent and not behaving normally and therefore are not readily available to be collected by the shotgun method employed in this study.

This is not implying that direct mortality is occurring under the operational spray program, although the possibility of direct mortality from operational

fenitrothion exists. **This study and previous studies (Lemon 1987, 1988) were not designed to assess songbird mortality, instead only to monitor ChE depression in collectable (live) birds. Monitoring ChE depression in live birds cannot assess mortality. Any conclusions relating the lack of songbird mortality observed in this study to operational fenitrothion spraying not causing mortality are unfounded.**

Field evidence (see Section 2.5.1) indicates that operational fenitrothion spraying can result in some songbirds becoming quite lethargic and quiescent, effectively removing them from the population being sampled. It is speculated that the most likely opportunity to sample severely affected individuals is as soon as possible following exposure, that is on the day of spray or the day following spray. With birds taking up to 30 days to fully recover from ChE depression and field and laboratory evidence of lethargic and quiescent behaviour in severely affected birds, the indication of "recovery" on the third day following the first spray is questionable based on this study. **The specific mechanisms involved in ChE depression and the consequences of individual songbird repsonse to fenitrothion exposure cannot be assessed using the methodology in this study.**

2.4.2 Biological Response

The relatively flat curves following the second spray may be explained based on expected behaviour of ChE depressed WTS as well. There appears to be an absence of the large decrease in ChE activity from Day 0 to Day 1 and a recovery as noted previously. However, there is a direct positive correlation (r=0.85) between the number of days between first and second sprays and the amount of ChE depression observed between Day 0 and Day 1 of the second spray.

As the amount of time (number of days) increased between first and second spray, there was a trend of an initial increase in ChE activity on the Day 0 collection. DF indicates the difference may be due to evening versus morning spray deliveries. Unfortunately this cannot be tested due to the opportunistic nature of spray application times. However, based on the review of the biological consequences of ChE depression, this increase in Day 0 ChE activity can be explained by the second spray delivery occurring later after the first spray delivery rather than sooner.

It is suggested that the lack of severe ChE depression observed in WTS following the second spray in Blocks 113 and 115 is because the blocks were sprayed relatively soon after the first spray. If the WTS are experiencing sublethal ChE depression resulting in lethargy, quiescence and anorexia, this behaviour could result in less feeding/foraging therefore ingesting less chemical. In addition, if the songbirds have become sensitive to the presence of fenitrothion chemical in the environment, they may respond by feeding less frequently, thereby ingesting less chemical (J. Fleming, pers. comm.)

However, by the time Blocks 109 and 108A were sprayed the ChE activity of WTS in those blocks had returned to a level above the critical level (i.e. not indicative of OP exposure). It would be expected that behaviour would then be more "normal" and foraging would intensify accordingly. The return to relatively normal behaviour may expose the WTS to more available fenitrothion through foraging and result in the increasing Day 1 ChE depression that was first observed following the first spray.

This explanation of the curves might be expected because the WTS in Blocks 109 and 108A would have more time to recover (enzyme synthesis) from first exposure.

These explanations for the observed differences in the data are presented only as a plausible interpretation of the data curves. It is not known how long WTS can survive if needed they experience a period of days without feeding. It is important that future scientific research should be directed towards addressing these differences. In addition, it is reasonable to expect that these levels of ChE depression will occur in future operational fenitrothion spray applications. **Future monitoring studies should address the issue of what these levels of ChE depression mean for the songbird populations in terms of breeding performance.**

2.5 OBSERVATIONS OF SONGBIRD ACTIVITIES

For the period of June 24 - July 3, all songbird singing was subdued because of cold and wet weather. Normally, this period should coincide with the tail end of the peak singing period for the majority of songbirds.

Singing was abundant at dawn on the clear mornings of July 4 and 5, but singing activity usually died down by 0800 h when temperatures increased. During this period, adult songbirds including WTS were frequently seen carrying food (insects) for young in nests.

The first fledged young birds were observed on July 6 in Block 109. There was one brood each of Northern Waterthrush (Seiurus noveboracensis) and Lincoln's Sparrow (Melospiza lincolnii). On July 7 and 8, 10 newly fledged families of Yellow-rumped Warblers (Dendroica coronata) were observed at various locations in and around the study area.

The first fledged brood of WTS was observed July 8 in Block 115. Over the next three or four days, there were frequent observations of WTS family groups, and by July 15, most pairs of adult WTS were accompanied by full sized juveniles.

There were some changes in the singing activity of adult WTS after the young left the nest. Male WTS singing was more sporadic; singing for briefer periods at dawn and during sunny periods of cloudy days. Male WTS response to imitations of its song was reduced in excitement level. Prior to the fledging of young, the male WTS usually responded by flying immediately to the imitator or singing back. With fledged young only approximately half of the male WTS responded in this way and when they did their interest waned quickly. This mannerism remained fairly constant until our departure from the field on July 28.

By late July, some juvenile WTS were probably feeding at least partially on their own but still stayed near the adults.

Breeding territories appeared to be maintained throughout the field study. This was determined because some male WTS were known by peculiar songs or ones singing from the preferred perches in the blocks until the end of the monitoring program. No more than four juveniles, the size of one brood, were seen in one place.

After July 10 sampling WTS occasionally became difficult if the day was hot, sunny and windy. Under these weather conditions, songbirds stay under cover out of the light and wind. This makes them difficult to find or bring out into the open. Despite these difficulties, prescribed daily sample sizes were obtained each day except on Block 115. Day 1 of the first spray (July 8) when field personnel collected one WTS short of the sample size due to hot, sunny and windy conditions.

2.5.1 Unusual Songbird Behaviour

There were three incidents of unusual songbird behaviour reported in the spray blocks following spray application. The three birds exhibiting unusual

behaviour were an adult male Black-throated Green Warbler (Dendroica virens) and an adult male Yellow-rumped Warbler in Block 108A (1" spray, Day 1), and an adult (sex unknown) Yellow-rumped Warbler in Block 115 (1" spray, Day 0).

1. Black-throated Green Warbler (BTGW) - One adult male. Spray Block 108A. Map Location VF856908. Line 18. 1" spray, Day 1 at 1330 h (30.5 h after spray delivery). Observed and reported by Mr. B. Mactavish.

"While walking along a woods road that was not accessible by truck, a small bird suddenly flushed up from near my feet. It flew low and weakly for 3-4 m and landed on the ground. I immediately recognized that something was wrong with the bird because it was so tame and acted as if it was weak. It was identified as an adult BTGW in high breeding plumage."

"It would attempt to flee when approached within 2-3 m which is abnormally close to any wild bird. It would flutter weakly 5-10 cm above the ground for 3 -7 m, before it attempted to hide in the grass or low vegetation. With caution I approached it to within 1 m and watched it carefully. Its eyes were bright, wide open and it seemed alert as it looked from side to side (normal behaviour for a warbler). Its overall plumage, bill, feet and legs appeared healthy and normal. The wings and tail quivered faintly but continuously. The wings were slightly drooped and the body feathers slightly puffed out. The body shook slightly and continuously and on a slower rhythm than the wings and tail. The bird sat on its feet and often shuffled them as if to gain balance. Several times it stretched out one wing flat out on the ground as if to hold itself upright. I observed it for the next 5 min and finally captured by throwing a shirt over it." The bird was then killed by squeezing its chest cavity and preserved whole in liquid nitrogen for later brain ChE analysis. The brain ChE activity for the BTGW was 5.6 units.

2. Yellow-rumped Warbler (YRW) - One adult male. Spray Block 108A, map location VF859908, Line 19. 1st spray, Day 1 at 1530 h (32.5 h after spray delivery). Observed and reported by Mr. B. Mactavish.

"Two hours after observing the BTGW, an adult male YRW was observed 250 m from the previously observed BTGW. It flew up from the ground about 1 m from where I was standing. It came to rest on a balsam fir branch about 8 m away and 3 m above the ground. When it landed it held its wings out for support on the branch for 15 sec before bringing them in against its body in a normal perching position. It remained there motionless for 5 min in a slightly fluffed out pose and sitting on its feet. It seemed to have trouble using its legs."

"It then flew 20 m to a taller tree where for the most of the next 15 min it remained in the same position crouched down on its legs and slightly fluffed out, occasionally putting out one wing on a branch as if to hold itself upright. It made three insect captures on the branches of the same tree. Each time fluttering weakly a short distance to catch the insect. It appeared awkward, clumsy and slow for a bird normally quick and agile. The bird disappeared when I was not looking."

The unusual behaviour of the two birds just described coincided with an unusual collection of dead and dying insects at 1000 h, 3 h after the finish of the 1st spray of Block 108A.

The insects were seen on a 150 m stretch of gravel road. They were unidentified but were 90% of one species - a black flying insect about 0.5 - 1.0 cm in length. Most were dead but in undamaged condition. A number were still alive lying on their sides or backs kicking their legs and trying to fly. One large dragon fly was on its back trying unsuccessfully to fly. There were butterflies and black flies flying unharmed through the area at

the time of observation. The area of dead insects was confined to the 150 m stretch of road. The vegetation was the same for approximately 200 m along the road before and after the kill. There were no water bodies nearby. The kill was near the crest of a steep hill. No other dead insects were noted elsewhere in the block despite searching.

We discussed our observations with the DF aerial supervisors on 108A. The spraying was started on the west side of the block on line 0 flying NW with a right echelon which means line 0-3 were sprayed on the first pass. Spraying conditions began to deteriorate on the northern half of line 24 with the planes flying NW with a right echelon meaning lines 24-27 were being sprayed. An east wind had increased enough to break up the spray cloud a little although the spray was seen by the spotter plane to be settling where it should below the spray planes. Spraying was terminated after lines 28-31 were done because the wind was still causing the cloud to break up.

The dead insects were found on line 20, the unusual behaving YRW and BTGW were observed on line 19 and 18 respectively. These are 450 - 800 m west of lines 24-27 where the wind was noticed to be breaking up the spray cloud. Although none of the spray from line 24-27 was seen to drift off the line, the wind was blowing toward lines 17-20. The location of the insects was on a steep hill and the two birds on the edge of the top of a relatively high hill.

3. Yellow-rumped Warbler - One adult, sex unknown. Spray Block 115, map location VF705807, Line 4, 1" spray, Day 0 at 1030 h. Observed and reported by Mr. D. Orr.

"The warbler was moving slowly through the grass and rolled over. I was able to reach down and touch the bird but I could not catch it. It flew up into the lower branch of a nearby tree."

3.0 CONCLUSIONS

The ChE activity of WTS was depressed on all days following fenitrothion spraying when compared to control samples. The ChE activity following the first and second spray application was similar to the results of the 1987 "intensive" spray block reported by Lemon (1988). A rapid depression in ChE activity immediately following the first spray appears to be followed by a ChE recovery on the third day following spray.

The ChE activity following the second spray application was not similar to that observed following the first spray. The results of second spray sampling produced a relatively flat curve indicating a prolonged sublethal depression of ChE activity. The rapid depression and relatively quick recovery observed after the first spray was not observed following the second spray.

The reason for the difference in the response curves after the first and second spray is not known. If the same response curve is expected after both spray applications, the severely affected WTS that generally appear on Day 1 following the first spray, are not evident following the second spray. A possible explanation is that all WTS are not exposed to the same amount of fenitrothion (as determined by ChE activity). Therefore any portion of the population experiencing severe exposure resulting in severe disability are available for sampling on Day 1 of the first spray but are lethargic/quiescent on subsequent sampling days and cannot be sampled using the collection methods in this study.

This raises the question, what is the fate of songbirds with sublethal ChE depression? **The fate of individual songbirds with sublethal ChE depression is not investigated in this study. This should be addressed through further study.** Based on this study, the WTS sampled on Day 1 of the first spray are, generally, the most severely affected samples. The Day 1

(first spray) sampling may be the only day following spray application when the severely exposed WTS can be collected. The other sampling days may represent individual WTS that, due to differences in behaviour, activity level or possibly being non-breeders or migrants, may not be exposed to (ingest) as much fenitrothion.

The percentage of songbirds showing exposure to fenitrothion in this study was approximately 58%. This value compares with previous studies where 70% were exposed in 1986 and 50% in 1987. The percentage of WTS with ChE activity below 50% of the control mean was approximately 9% in this study. This amount is reduced considerably from 38% in 1986 and 17% in 1987. The distribution of severely affected (ChE activity depressed $\geq$50%) appeared more evenly distributed amongst the monitored spray blocks in this study compared to the 1987 monitoring study. The reduced number of severely affected WTS and the more even distribution may be a result of differences in application methodology (small aircraft/atomizers vs. large aircraft/boom and nozzle).

There was a trend of increased ChE recovery on Day 0 of the second spray as the number of days between first and second spray applications increased. There was also an increase in the depression in ChE activity between Day 0 and Day 1 of the second spray as the number of days between first and second spray increased.

Based on the review of the pertinent literature concerning the effects of ChE depression in altricial songbirds and the magnitude and frequency of ChE depression observed in this study and Lemon (1987, 1988), there is cause for serious concern over songbird safety.

The dissimilarity between the spray block ChE activity curves following the two spray applications raises the question as to whether the expected rapid "recovery" typically observed following the first spray is actually a true population recovery. It is postulated, in this study, that it may be an sampling artifact produced by songbird behaviour associated with severe ChE depression and sampling bias present in the collection methodology.

The last several year's monitoring studies have pieced together a comprehensive, integrated effects monitoring study. In 1986 one spray block was monitored, followed in 1987 with six spray blocks being monitored with one "intensive" monitored spray block. In 1988 four spray blocks were monitored with a thorough investigation of both operational sprays on songbirds brain ChE activity. Based on these integrated studies, and a thorough review of the biological consequences of ChE depression in birds, the existing use of fenitrothion should be reviewed in terms of songbird safety.

4.0 RECOMMENDATIONS

The recommendations made by Lemon (1988) stressed the need for population level effects monitoring of songbirds in addition to the individual level effects monitoring conducted in this study. **This recommendation has not been addressed by the Department of Environment and Lands.** Over the past several years, the Department of Forestry has put together an adequate integrated effects monitoring program to investigate the effects of operationally applied fenitrothion on individual songbirds as determined by brain ChE activity.

The following recommendations relate to songbird safety and are based on the results of this study, results of the previous related songbird monitoring studies reported by Lemon (1987, 1988), a literature review of the biological consequences of ChE depression:

1) The fenitrothion application methodology used in this year's monitoring study appears to have an overall less severe effect on forest songbirds as determined by the percentage of ≥50% ChE depressed WTS and the relatively uniform distribution of the samples. If fenitrothion is to be used in the future, small aircraft using rotary atomizers are recommended for the spray application, based on this study.

2) Future fenitrothion monitoring efforts should be designed to address biological concerns over the fate of severely affected individual songbirds and the effect of sublethal ChE inhibition resulting from chronic exposure on songbirds reproductive performance.

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6.0 PERSONAL COMMUNICATIONS

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APPENDIX A

ORGANOPHOSPHORUS PESTICIDES AND ChE DEPRESSION

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ORGANOPHOSPHORUS PESTICIDES AND ChE DEPRESSION

A1 ACTION OF ORGANOPHOSPHORUS PESTICIDES

Fenitrothion, like other organophosphorus (OP) pesticides, blocks synaptic transmissions in the cholinergic parts of the nervous system (Fleming and Grue 1981). The ChE inhibitors permit excessive acetylcholine accumulation at nerve synapses thus disrupting or blocking nerve impulse transfer (Corbett 1974). Signs of ChE inhibition in birds resulting from exposure to OP pesticides include myosis, salivation, lacrimation (muscurinic effects), muscle twitching, dyspnoea (breathing difficulties), paralysis and clonic convulsions (nicotinic effects) (Zinkl et al. 1977). Other signs are anorexia and lethargy (Pope and Ward 1972).

A2 SAMPLING BIAS IN ChE DETERMINATION METHODS

There is a bias involved when using brain ChE determination to assess exposure of birds to OP pesticides. The collection procedure results in the potential to collect healthier more conspicuous individuals from the exposed population (Mineau and Peakall 1987). This bias would tend to underestimate the extent of OP exposure of the population in general when assessed by ChE determination.

Sublethal OP exposure effects causing aberrant behaviour (e.g. clonic convulsions, paralysis, dyspnoea, lethargy) resulting in physical malaise or death may preclude such affected individuals from readily being collected in the field. Birds with severe sublethal ChE depression may be less likely to respond to "pishing" or song imitations temporarily until ChE activity recovers to a level where "normal" functioning occurs (length of time not known). In addition, such severely affected individuals may be restricted to ground and

low lying vegetation (see Section 3.5.1) and would only be found by chance.

A3 BIOLOGICAL CONSEQUENCES OF ChE DEPRESSION IN ALTRICIAL SONGBIRDS

Depression in brain ChE activity and the corresponding biological consequences are not well known. Researchers have arbitrarily determined that a songbird showing ≥50% ChE depression could be considered to be in a potentially life-threatening situation (Ludke et al. 1975; Zinkl et al. 1980). Obviously birds in this study that exhibited brain ChE depressions ≥50% of that observed in the control sample were not dead, otherwise they could not have been collected for this study. The results of this study should provide an indication of the level of exposure to fenitrothion experienced by individual WTS in the treated areas with the sampling bias (Section 2.2) taken into consideration.

Birds appear to be exposed to OP pesticides through ingestion of contaminated food and through dermal (including respiratory) contact (Finley 1965; Fowle 1972; White et al. 1979, 1982). Nestlings appear to be exposed by ingesting contaminated food items supplied by parent birds (Robinson et al. 1988). Applications of these pesticides often occur during the seasonal peak in avian abundance and reproductive activity.

The potential for operationally applied OP pesticides, including fenitrothion to cause direct mortality to birds is known (Seabloom et al. 1973; Grue et al. 1983; Hardy and Stanley 1984). The concern during the breeding season is that aberant behaviour induced by sublethal ChE depression in adult birds during egg laying, egg incubation, brood rearing or in the postfledging period may result in decreased breeding performance. Other biological concerns relate to the sublethal effect of fenitrothion on nestlings and newly fledged songbirds.

A3.1 Results of Field Studies

Grue et al. (1982a, 1982b) dosed European Starlings (Sturnus vulgaris) with enough ChE inhibitors to produce an average ChE depression of approximately 50% when compared to unexposed controls. They observed that female Starlings with such a sublethal depression in ChE activity made significantly ($P<0.05$) fewer sorties to feed their young and remained away from their nest boxes for longer periods of time than control birds (Grue et al. 1982a). Their results indicate that parental care may be significantly reduced in songbirds receiving sublethal ChE depression following chronic (repeated) exposure to ChE inhibitors. Grue et al. (1982b) reported weight loss in OP dosed adult female Starlings due to anorexia and lethargy. In addition, Grue et al. (1982a) reported significantly greater weight loss in nestlings of OP dosed female Starlings compared to control females. This indicates that OP dosed females did not compensate for fewer feeding trips by collecting more food per trip.

Nestlings of altricial birds may not gain their full adult complement of ChE until well after fledging. Grue et al. (1981) reported that brain ChE in nestling Starlings was age dependant and that ChE levels of 18 day old nestlings (1-3 days from fledging) were only 70% of those found in adults. If brain ChE developed at a constant rate, fledgling Starlings would reach their adult complement of brain ChE at approximately 28 days (Grue et al. 1981). Age related sensitivity of altricial songbirds has not been investigated. However, since nestling Starlings have less ChE per gram of brain tissue than adults, it is possible that less exposure to ChE inhibitors would be required to induce behavioural effects or mortality in young altricial species (Grue et al. 1981). Robinson et al. (1988) reported a significant positive relationship between brain ChE depression in adult Starlings exposed to OP pesticides and their nestlings. Although no significant difference in egg hatchability was observed between Starling nests in exposed and control areas, the number of nestlings fledged in the ChE inhibitor treated field was significantly lower than

the control field (Robinson et al. 1988). Robinson et al. (1988) found that the the degree of ChE depression in nestlings (<20%) could be significant when the age of the bird is considered and that the nesting success of ChE inhibited birds could be significantly affected by increased mortality and reduced fitness of surviving chicks.

The nestlings of altricial birds attract and stimulate feeding adults by gaping (wide-open mouths), head waving and by their cries (Welty 1982). Since the foraging activity of parents is stimulated by the begging intensity of the young, OP induced alteration in begging and gaping responses may alone significantly reduce energy intake (Grue et al. 1982a).

Perrins (1963, 1965) indicated a strong positive correlation between fledging weight and post-fledging survival in passerines. Heavier young at the time of fledging may have far greater fat reserves to better enable them to survive the post-fledging period when they are learning to forage (Perrins 1965; Crossner 1977). Garnett (1981) suggested that the relationship between the proportion of aggressive interactions that an individual dominates and its body size accounts for the relationship between postfledging survival and body size. In contrast, however, Stromborg et al. (1988) could not demonstrate any relationship between body mass at fledging and postfledging survival in Starlings whether they had been exposed to a single dose of an OP pesticide as a nestling or not.

Pearce and Busby (1980) reported the results of a study in which WTS were monitored for reproductive parameters and other WTS were collected for brain ChE determination. Both groups were subjected to the same fenitrothion spray. In the study, mean ChE depression in exposed birds was 42% after the first spray and 29% after the second spray (Pearce and Busby 1980). They found that the reproductive success was severely reduced in birds monitored in the sprayed area. Young fledged from only 11.6% of the eggs laid in nests in

sprayed areas compared to young fledgling from 58.3% of eggs laid in nests from unsprayed areas (Pearce and Busby 1980).

Spray et al. (1987) investigated the effects of fenitrothion spray applications on forest songbirds and reported results indicating little or no effect on songbirds from sprayed plots compared to unsprayed plots. Spray et al. (1987) did not find any significant differences in clutch size or brood size between sprayed and unsprayed areas. However, they reported a trend for slower growth resulting in a lower final nestling weight in sprayed areas (Spray et al. 1987). Brain ChE analysis was not performed in conjunction with this study.

A3.2 Results of Laboratory Studies

Experimental conditions in laboratories create an artificial situation (artificial environment, food and water ad libitum, no predators) that does not duplicate field conditions. Although there is concern over direct mortality of adult songbirds resulting from operational OP applications, this is not the only issue as far as adverse effects on forest songbirds is concerned. The debilitating effects of chronic exposure to OP pesticides causing sublethal ChE depression that may result in reduced reproductive success of adults and reduced fitness of nestlings is also a concern.

Laboratory studies aimed at determining the biological consequences of fenitrothion and other OP pesticides on birds have been conducted. These studies indicate that if the bird survives initial exposure, they will recover (Miyamoto 1977; Fleming 1981; Fleming and Bradbury 1981; Fleming and Grue 1981; Holmes 1988).

It is important to note that in the intitial dosing, some birds do die in spite of the fact that they were given the same dose as those that survived. In addition, the results of these studies were assessed in the birds surviving the

initial exposure and not included in the results were the mortalities incurred.

Miyamoto (1977) reported no noticeable differences in terms of egg production, egg weight and quality of eggs of Bobwhite Quail (Colinus virginianus) in the laboratory between control birds and ones fed a diet containing 3 ppm and 10 ppm fenitrothion. However, the mortality of these fenitrothion fed birds increased over that observed in control birds by 33% (10 ppm fenitrothion feed) and 133% (3 ppm fenitrothion feed) (Miyamoto 1977). Fleming and Bradbury (1981) and Holmes (1988) also report mortality of laboratory test birds having been feed diets containing organophosphorus pesticides. Mortality of birds and other wildlife from operational spray programs has been reviewed and reported in Grue et al. (1983).

Holmes (1988) conducted laboratory studies on the effects of single acute oral doses of fenitrothion on the reproductive success of Zebra Finches (Poephila guttata). Paired (although separated) Zebra Finches were grouped into control and treatment groups. Treatment groups were dosed one time only with either 1.04 mg/kg (body mass) or 3.80 mg/kg (body mass) fenitrothion. Pairs of Finches were kept apart until the day prior to dosing and subsequent clutch size, hatchability, brood size and number fledged of each pair was recorded.

Although the results were not statistically significant, distinct trends were observed associated with increased fenitrothion dosage (Holmes 1988). These were increased mortality, a decrease in activity, a decrease in production, a shortening in breeding cycle and a reduction in the number of successful nests (10 out of a possible 16 in control compared to 5 out of 16 in 3.80 mg/kg dosed pairs). It is questionable whether or not Holmes (1988) could demonstrate an effect on reproductive success in his experiment. The brain ChE of adult Finches that were dosed with 1.04 mg/kg and 3.80 mg/kg fenitrothion recovered to within 80% of control by 4.0 and 6.5 days respectively. Mean number of days to hatch for the two treated groups were

25.8 and 23.6 days respectively (Holmes 1988). With brain ChE levels of treated Finches recovering to levels not being indicative OP pesticide exposure (Zinkl et al . 1980) prior to egg hatching, Holmes (1988) should not have been able to detect a good reproductive success effect under laboratory conditions. Holmes (1988) may have only been able to detect the effect of his dosing procedure on behaviour and egg viability.

A3.3 Summary

The previous brief review of recent field and laboratory studies can be summarized in the following points:

i) exposure to OP pesticides, including fenitrothion, can result in songbird mortality

ii) sublethal ChE depression is reversible

iii) sublethal ChE depression in adult birds produced a trend toward reduced reproductive performance in the laboratory

iv) sublethal ChE depression in Starling nestlings (exposed at 16 days) did not adversely affect nestling mass at fledging or postfledging survival

v) nestlings of altricial songbirds have lower concentrations of ChE than adults and are exposed to OP pesticides primarily through ingestion of contaminated food from adults

vi) research indicates that sublethal ChE depression in nestling atricial songbirds may result in reduced weight gain and reduced adult reproductive success

APPENDIX B

Environmental Monitoring Program
1988 Hemlock Looper Spray Program

Terms of Reference

May 15, 1988

1. Introduction

The Department of Forestry has identified 139 blocks comprising a total area of 88,555 hectares to be treated with fenitrothion (Folithion[R] and/or Sumithion[R]) and Bt (Dipel R 176) in response to predicted outbreaks of the eastern hemlock looper (Lambdina fiscellaria fiscellaria). As a stipulation of their 1988 operator's licence the Department of Forestry has been directed to undertake a monitoring study that examines cholinesterase (ChE) levels in white-throated sparrows (Zonotrichia albicollis) following treatment of spray blocks using fenitrothion formulations.

One of the major recommendations arising out of the 1987 monitoring study was the need to address the concern about the ability of songbirds to recover from a repeated exposure to fenitrothion formulations under operational spray conditions (S. Fudge and Associates, 1988). Consequently, the objective behind the 1988 monitoring program will be to determine the magnitude and pattern of brain ChE recovery in white-throated sparrows after two (2) applications of fenitrothion formulations applied at a rate of 210 gm ai/ha.

Experimental Design

1. Monitoring activities will take place on four (4) spray blocks. The exact location of monitored blocks will be determined by mutual agreement between the Contractor and the Department of Forestry.

2. Sample size of each sample will be determined using standard statistical practice as illustrated in Box 9:13 of Sokal and Rohlf (1981). Variance estimates are to be determined using data from past monitoring studies in Newfoundland. Desired sensitivity, power, and level of significance are to be set in consultation with the Scientific Authority prior to commencement of the field program. A full sample Will be collected on each day of the following schedule:

A. Blocks using two (2) applications of fenitrothion - formulations applied at a rate of 210 g ai/ha:

   1. day of first spray
   2. one day after spray
   3. three days after spray
   4. day of second spray
   5. one day after spray
   6. three days after spray
   7. five days after spray
   8. seven days after spray

3. Birds will be shot with one and three-quarter inch or two and one-half inch shotshells with #10 and #9 shot in a .410 calibre shotgun or suitable substitute. After shooting specimens will be appropriately labelled, prepared, and stored for subsequent brain ChE determination by the Canadian Wildlife Service in Fredericton, New Brunswick. The Contractor shall be responsible for all details regarding preparation and shipment of samples.

4. One (1) sample set will be collected per monitored spray block prior to that block being treated with fenitrothion formulations.

5. Birds will be collected widely throughout the blocks. No birds will be collected within 200 m of a sample block border.

6. For each bird collected the following parameters will be noted:

   A. Time of post-spray collection
   B. Whether the individual was a juvenile or not

C. Forest canopy characteristics at point of collection (a scale of 1 to 5 is to be used; 1 being open, 5 being closed canopy).

Report

A report will be made available by deadlines specified in the Payment Schedule and shall include, but not be restricted to, the following sections:

1. Abstract/Executive Summary: A brief discussion of the study, highlighting the findings and their significance.

2. Introduction: Will include a discussion of the rationale behind using brain ChE levels to examine organophosphate exposure. Will also include a clear statement on the objective of the study.

3. Materials and Methods: Will include the following discussions:

   A. Techniques used to collect birds
   B. Analytical techniques used in ChE assay
   C. Location and description of spray blocks studied
   D. Spray application details
   E. Relevant meterological parameters at times of collection

4. Results: Will include the following:

   A. Data presented in tabular analor graphical format
   B. Appropriate statistical treatment of data
   C. Rationale behind statistical treatments
   D. Comments on data (anomalies, trends, etc.)

5. Discussion: Will include a discussion of the data in light of the scientific literature on the subject. An examination of the biological significance of ChE inhibition will also be included in this section. Other points of discussion will include, but not be restricted to, the following:

   A. Conclusions of the Study
   B. Recommendations

6. Bibliography: Will include a summary of all literature examined for the Study.

APPENDIX C

White-throated Sparrow collection areas, ChE values, forest cover and relevant meteorologic parameters at time of collection

SPRAY BLOCK 108A

Figure C1: Distribution of White-throated Sparrow collection in Block 108A

Table C1: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Figure C-1

DISTRIBUTION OF WHITE-THROATED SPARROW COLLECTION IN BLOCK 108A

LEGEND

First Spray	Second Spray
1 Day of spray	4 Day of Spray
2 Day after spray	5 Day after spray
3 Third day after spray	6 Third day after spray

——— Spray line
····· Shut off

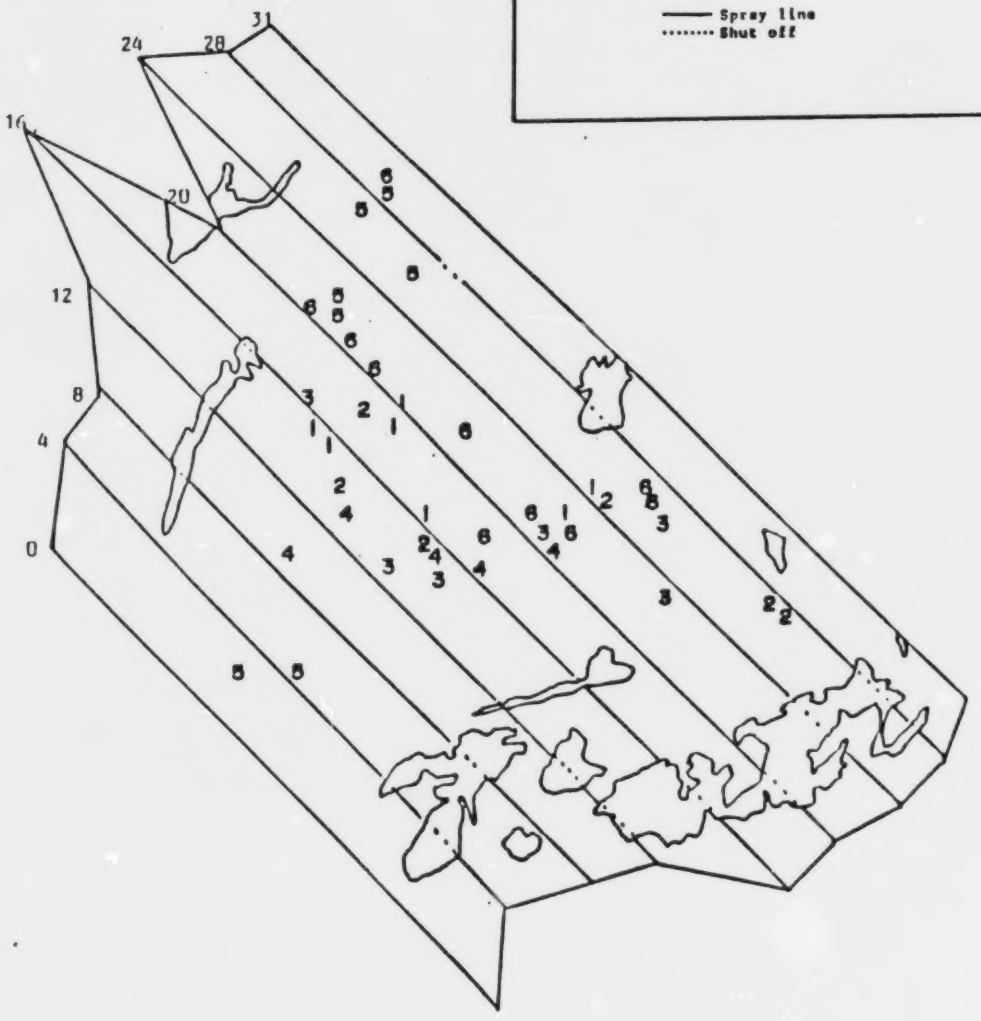

Table C1: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

BLOCK 108A

Collection Day	ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather: Sky	Weather: Temp. (°C)	Weather: Wind (km/hr)
First Spray								
Day 0	20.4	July 7	Fir & Birch	1-3 m	50%	Clear	17	0-5
Day 0	14.5	July 7	Fir & Birch	1-3 m	50%	Clear	20	0-5
Day 0	15.2	July 7	Fir	1-3 m	70%	Clear	20	0-5
Day 0	21.6	July 7	Birch & Fir	3-7 m	80%	Clear	24	5-10
Day 0	18.9	July 7	Birch	3-5 m	80%	Clear	24	10
Day 0	28.0	July 7	Thinned Fir	3-5 m	40%	Clear	24	15
Day 1	21.2	July 8	Thinned Fir	3-5 m	30%	Rain	10	0-5
Day 1	22.8	July 8	Fir & Birch	4-6 m	70%	Light Rain	10	0-5
Day 1	18.1	July 8	Fir & Birch	2-3 m	90%	Light Rain	10	0-5
Day 1	17.4	July 8	Thinned Fir	4-5 m	50%	Overcast	12	5
Day 1	6.8	July 8	Fir	1 m	40%	Overcast	12	Light
Day 3	24.5	July 10	Spruce & Fir	10 m	70%	Fog	15	5
Day 3	23.6	July 10	Fir	4 m	60%	Fog	15	5
Day 3	20.4	July 10	Thinned Fir	3-4 m	50%	Overcast	15	10
Day 3	13.5	July 10	Thinned Fir	3-4 m	50%	Overcast	15	10
Day 3	16.0	July 10	Thinned Fir	4 m	40%	Overcast	15	Moderate
Day 3	12.5	July 10	Birch & Fir	1-4 m	50%	Partial Cloud	17	10
Second Spray								
Day 0	25.3	July 24	Fir	2-4 m	60%	Overcast	18	Light
Day 0	23.6	July 24	Fir	8-10 m	70%	Clear	15	Light
Day 0	25.3	July 24	Spruce & Fir	10 m	50%	Clear	15	Light
Day 0	19.3	July 24	Fir	2-4 m	70%	Clear	15	Light
Day 0	16.7	July 24	Birch	3 m	60%	Sunny	20	15
Day 0	18.1	July 24	Birch	3 m	60%	Sunny	20	15
Day 1	12.5	July 25	Fir	3-4 m	50%	Overcast	14	Light
Day 1	18.5	July 25	Birch & Fir	2-4 m	50%	Overcast	14	Light
Day 1	18.5	July 25	Fir	3-10 m	70%	Overcast	14	Light
Day 1	11.8	July 25	Thinned Fir	2-4 m	40%	Overcast	14	Light
Day 1	20.4	July 25	Fir	4-5 m	30%	Overcast	14	Light
Day 1	12.5	July 25	Birch	2 m	50%	50% Cloud	16	Light
Day 3	12.8	July 27	-	1-3 m	10%	Overcast	12	0-5
Day 3	18.9	July 27	Birch & Fir	4-8 m	70%	Partial Cloud	12	Light
Day 3	9.9	July 27	Fir	15-20 m	70%	Overcast	12	Light
Day 3	15.2	July 27	Birch	5-6 m	70%	Overcast	12	Light
Day 3	15.6	July 27	-	2 m	10%	Overcast	18	Light
Day 3	15.2	July 27	-	7 m	40%	Overcast	20	Light
Day 3	18.1	July 27	Birch	3 m	30%	Overcast	20	Light
Day 3	21.2	July 27	Spruce & Birch	3-5 m	30%	Partial Cloud	20	Light
Day 3	16.3	July 27	-	4 m	80%	Partial Cloud	22	Light
Day 3	18.1	July 27	Birch & Fir	5 m	90%	Overcast	20	Light

[1] Fir - Abies balsamea
Birch - Betula papyrifera
Tamarack - Larix laricina
Willow - Salix sp.
Spruce - Picea sp.
Alders - Alnus sp.
Maple - Acer sp.

[2] Light - 0-5
Moderate - 5-10
Strong - >10

SPRAY BLOCK 108B

Figure C2: Distribution of White-throated Sparrow collection in Block 108B

Table C2 : White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Figure C.8

DISTRIBUTION OF WHITE-THROATED SPARROW COLLECTION IN BLOCK 1088

LEGEND

First Spray
1 Day of spray
2 Day after spray
3 Third day after spray

—— Spray line
····· Shut off

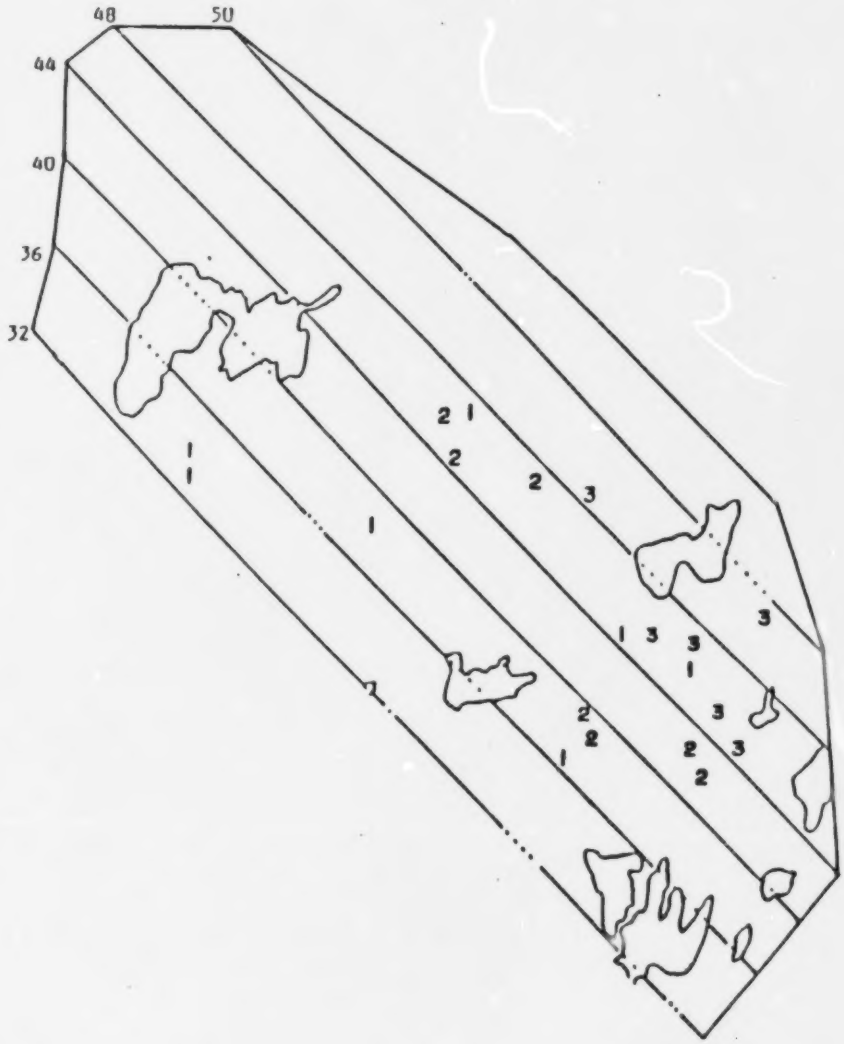

Table C2: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

BLOCK 108B

Collection Day	ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather		
						Sky	Temp. (°C)	Wind (km/hr)[2]
First Spray								
Day 0	11.8	July 13	Birch	4 m	50%	Rain	10	Light
Day 0	27.5	July 13	Fir & Birch	3 m	50%	Overcast	10	5
Day 0	16.0	July 13	Birch & Fir	4-6 m	60%	Overcast	10	0-5
Day 0	18.9	July 13	Thinned Fir	3-5 m	40%	Overcast	10	0-5
Day 0	24.1	July 13	Fir	2 m	60%	Overcast	10	Light
Day 0	26.2	July 13	Birch	2 m	50%	Overcast	10	Light
Day 0	19.7	July 13	-	3 m	30%	Overcast	10	Light
Day 1	16.3	July 14	Birch & Fir	4 m	70%	Clear	8	0-5
Day 1	22.4	July 14	Birch & Fir	4 m	60%	Clear	10	Light
Day 1	19.7	July 14	Fir	10-12 m	70%	Clear	13	5
Day 1	26.2	July 14	-	3 m	70%	Clear	12	Light
Day 1	14.5	July 14	-	2-3 m	85%	Clear	12	Light
Day 1	26.2	July 14	Fir & Birch	3 m	70%	Partial Cloud	10	Light
Day 1	26.2	July 14	Fir & Birch	3 m	70%	Partial Cloud	16	Light
Day 3	14.5	July 16	Fir	6-8 m	80%	Sunny	13	0-10
Day 3	24.9	July 16	Fir	1-3 m	5%	Sunny	13	0-10
Day 3	18.9	July 16	Fir & Spruce	6-10 m	100%	Sunny	13	0-10
Day 3	23.2	July 16	Thinned Fir	3-5 m	50%	Sunny	13	0-10
Day 3	25.7	July 16	Fir	5-8 m	70%	Sunny	13	0-10
Day 3	18.1	July 16	Thinned Fir	5-8 m	50%	Sunny	13	10-13

[1] Fir - *Abies balsamea*
Birch - *Betula papyrifera*
Tamarack - *Larix laricina*
Willow - *Salix* sp.
Spruce - *Picea* sp.
Alders - *Alnus* sp.
Maple - *Acer* sp.

[2] Light - 0-5
Moderate - 5-10
Strong - >10

SPRAY BLOCK 109

Figure C3: Distribution of White-throated Sparrow collection in Block 109

Table C3 : White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Figure C.8

DISTRIBUTION OF WHITE-THROATED SPARROW COLLECTION IN BLOCK 109

LEGEND

First Spray
1 Day of spray
2 Day after spray
3 Third day after spray

Second Spray
4 Day of spray
5 Day after spray
6 Third day after spray
7 Fifth day after spray
8 Seventh day after spray

—— Spray line
····· Shut off

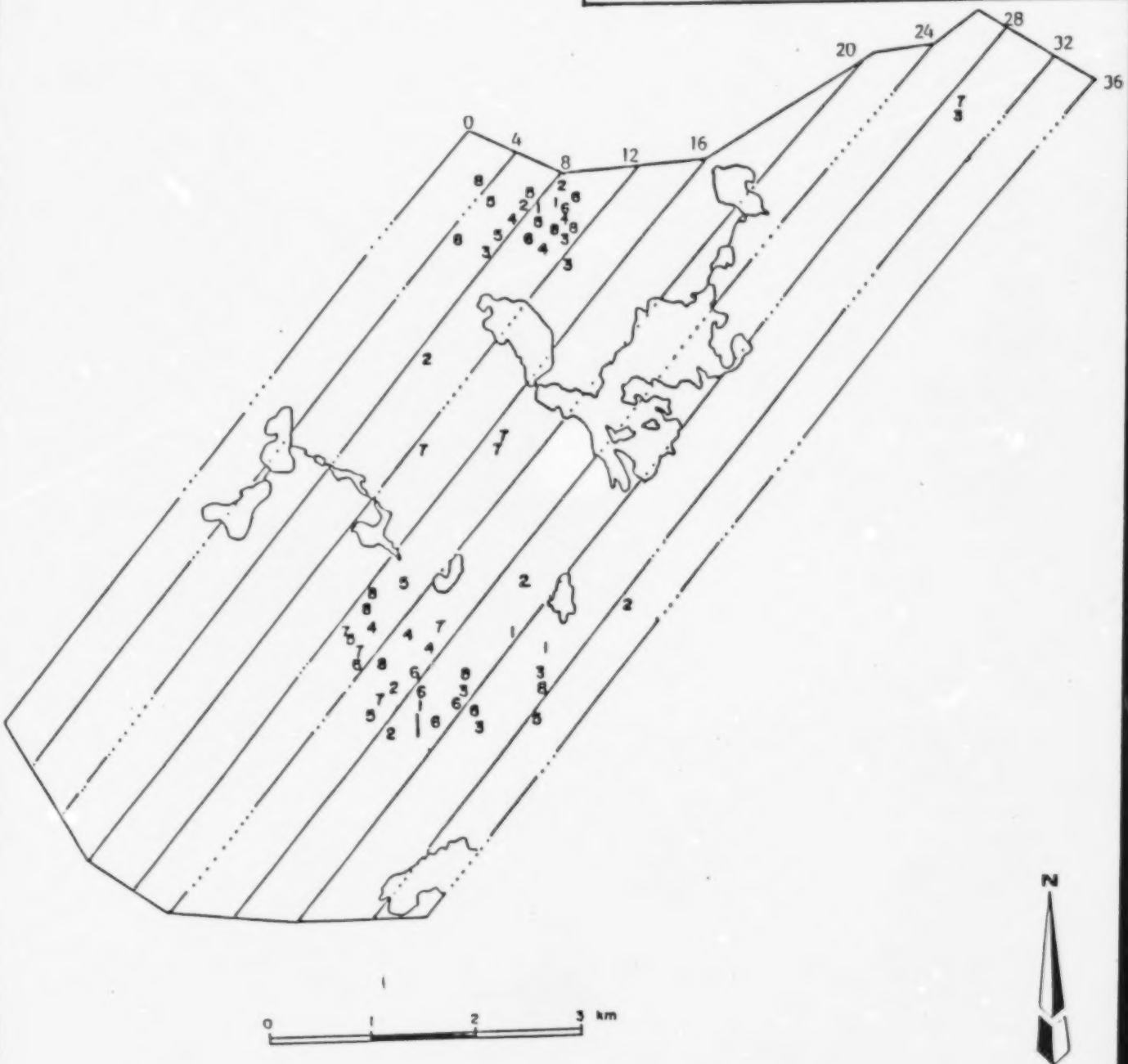

Table C3: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

BLOCK 109

Collection Day	ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather: Sky	Weather: Temp. (°C)	Weather: Wind (km/hr)[2]
First Spray								
Day 0	26.2	July 5	Spruce	5 m	30%	Clear	15	15
Day 0	22.0	July 5	Tamarack	2 m	20%	Clear	15	15
Day 0	27.1	July 5	Birch & Fir	3 m	80%	Clear	20	15
Day 0	19.7	July 5	Fir	1-3 m	30%	Clear	20	15
Day 0	26.6	July 5	Fir	2-3 m	40%	Clear	24	15
Day 0	27.1	July 5	Thinned Fir	2-3 m	30%	Clear	24	15
Day 0	24.5	July 5	Fir	2 m	30%	Clear	24	15
Day 1	23.6	July 6	Thinned Fir	3-4 m	40%	Overcast	12	5
Day 1	9.2	July 6	Spruce & Fir	2-6 m	70%	Overcast	12	5
Day 1	14.5	July 6	-	2-3 m	30%	Overcast	12	Light
Day 1	11.2	July 6	-	5-7 m	20%	Overcast	12	Light
Day 1	21.2	July 6	Fir	2-10 m	90%	Overcast	18	Light
Day 1	14.5	July 6	Aldera	2-4 m	95%	Overcast	15	Light
Day 1	18.1	July 6	Fir	3 m	40%	Overcast	15	2-5
Day 3	22.8	July 8	-	3 m	20%	90% Cloud	12	5
Day 3	22.4	July 8	Tamarack	2 m	20%	Overcast	10	Light
Day 3	16.7	July 8	-	3 m	85%	Overcast	10	Light
Day 3	13.8	July 8	Thinned Fir	3 m	40%	Overcast	10	0-5
Day 3	18.9	July 8	Aldera	1 m	25%	Overcast	12	0-5
Day 3	22.8	July 8	Fir	5-8 m	70%	Overcast	15	Light
Day 3	27.1	July 8	Fir	2-3 m	60%	Overcast	15	Light
Second Spray								
Day 0	19.7	July 19	Fir & Birch	3-4 m	50%	Fog	10	Light
Day 0	24.5	July 19	Birch & Fir	2-4 m	50%	Overcast	10	Light
Day 0	25.3	July 19	Birch & Fir	5-15 m	60%	Overcast	10	Light
Day 0	26.6	July 19	-	3 m	20%	Clear	22	Light
Day 0	20.8	July 19	-	4 m	40%	Overcast	14	Light
Day 0	18.1	July 19	-	3 m	30%	Overcast	14	Light
Day 1	22.8	July 20	Birch & Fir	3-5 m	50%	Overcast	14	Light
Day 1	18.5	July 20	Fir	5-10 m	70%	Light Rain	14	0-5
Day 1	15.2	July 20	Fir	7-8 m	100%	Showers	14	0-5
Day 1	18.1	July 20	-	1-3 m	5%	Overcast	8	0-5
Day 1	25.3	July 20	-	2-3 m	60%	Overcast	10	0-5
Day 1	15.2	July 20	-	6 m	30%	Overcast	12	Light
Day 1	23.6	July 20	-	5 m	20%	Overcast	12	Light
Day 3	12.5	July 22	Spruce	2-4 m	50%	Partial Clouds	21	20
Day 3	22.0	July 22	Fir	5 m	100%	Overcast	10	Light
Day 3	24.5	July 22	Fir	3-4 m	70%	Overcast	10	Light
Day 3	18.9	July 22	Fir	2 m	30%	30% Cloud	17	Moderate
Day 3	17.0	July 22	Fir	2 m	20%	30% Cloud	20	Moderate
Day 3	24.5	July 22	-	4 m	70%	Clear	24	Strong
Day 3	23.6	July 22	-	7 m	90%	Clear	24	Strong
Day 3	20.4	July 22	-	7 m	90%	Clear	24	Strong
Day 3	10.5	July 22	Fir	7 m	80%	60% - 70% Cloud	20	Moderate
Day 5	24.5	July 24	-	6 m	50%	Clear	24	Light
Day 5	13.2	July 24	Fir	2-3 m	30%	Clear	12	Light
Day 5	18.9	July 24	Fir	2-3 m	30%	Clear	12	Light
Day 5	16.7	July 24	-	4 m	70%	Clear	24	Light
Day 5	21.6	July 24	-	9 m	85%	Overcast	20	Light
Day 5	17.0	July 24	Fir	6 m	70%	Overcast	22	Light
Day 5	17.4	July 24	Fir	7 m	80%	Overcast	22	Light
Day 5	24.5	July 24	Fir	8 m	100%	Overcast	23	Light
Day 7	19.7	July 26	Spruce	4-5 m	60%	Clear	20	Light
Day 7	17.4	July 26	Spruce	10-15 m	60%	Clear	20	Light
Day 7	25.3	July 26	Fir	2-3 m	40%	Clear	20	Light
Day 7	21.2	July 26	Spruce	5-6 m	60%	Clear	22	10
Day 7	16.7	July 26	-	7 m	40%	Clear	20	Light
Day 7	20.4	July 26	Birch	1 m	40%	Clear	18	Light
Day 7	25.8	July 26	Birch	1-2 m	20%	Clear	17	Light
Day 7	28.0	July 26	Grass	0	0%	Clear	22	Light
Day 7	28.4	July 26	Fir	2 m	30%	Clear	25	Light

1 Fir - *Abies balsamea*
Birch - *Betula papyrifera*
Tamarack - *Larix laricina*
Willow - *Salix* sp.
Spruce - *Picea* sp.
Aldera - *Alnus* sp.
Maple - *Acer* sp.

2 Light - 0-5
Moderate - 5-10
Strong - >10

SPRAY BLOCK 113

Figure C4: Distribution of White-throated Sparrow collection in Block 113

Table C4 : White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Figure C. 4

DISTRIBUTION OF WHITE-THROATED SPARROW COLLECTION IN BLOCK 113

LEGEND

First Spray	Second Spray
1 Day of spray	4 Day of Spray
2 Day after spray	5 Day after spray
3 Third day after spray	6 Third day after spray
	7 Fifth day after spray
	8 Seventh day after spray

——— Spray line
····· Shut off

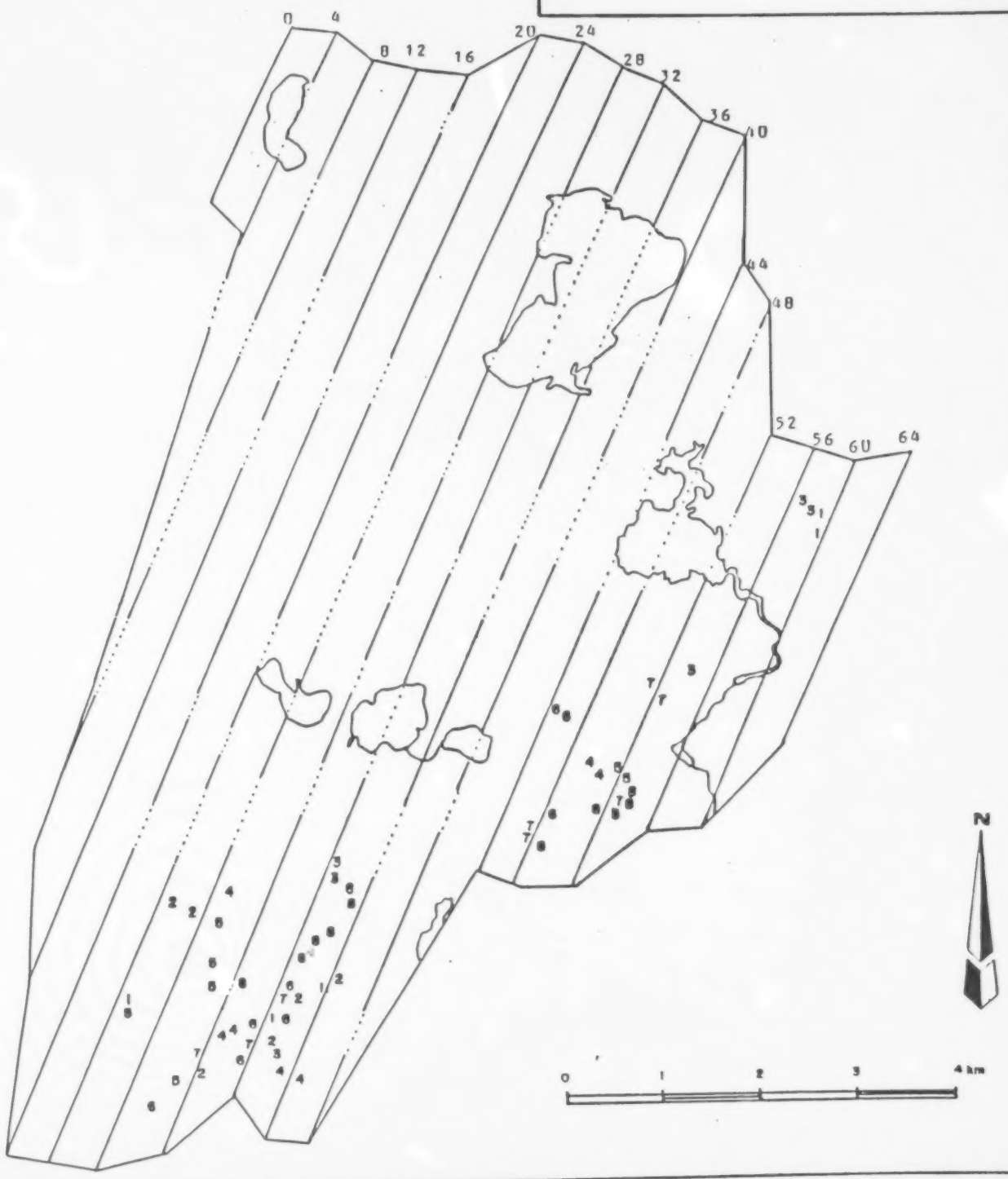

Table C4: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

BLOCK 113

Collection Day	ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather: Sky	Weather: Temp. (°C)	Weather: Wind (km/hr)[2]
First Spray								
Day 0	25.3	July 15	Deciduous & Fir	2 m	45%	Rain	10	Light
Day 0	21.2	July 15	Spruce & Fir	4-5 m	75%	Rain	10	Light
Day 0	24.5	July 15	-	3-4 m	40%	Rain	11	Light
Day 0	21.6	July 15	-	2 m	20%	Rain	11	Light
Day 0	16.7	July 15	Fir	3 m	20%	Light Rain	11	Light
Day 0	22.4	July 15	Fir & Birch	3-5 m	100%	Light Rain	10	Light
Day 1	18.1	July 16	Fir	4 m	30%	Clear	14	Light
Day 1	9.9	July 16	Fir	4 m	10%	Clear	15	Light
Day 1	21.2	July 16	Fir	1-2 m	10%	Clear	20	Moderate
Day 1	13.5	July 16	Fir	3 m	15%	Clear	20	20
Day 1	22.0	July 16	-	3 m	80%	Clear	18	20-30
Day 1	22.0	July 16	Fir	3 m	90%	Clear	20	Strong
Day 3	26.6	July 18	Fir	5-10 m	50%	Clear	20	10
Day 3	20.8	July 18	Birch & Fir	8-10 m	60%	Clear	20	15
Day 3	25.3	July 18	Spruce	3-5 m	50%	Clear	21	15
Day 3	20.0	July 18	Alder	1-3 m	100%	Sunny	18	0-15
Day 3	22.0	July 18	Alder	1-3 m	100%	Sunny	18	0-15
Day 3	14.5	July 18	Birch	2-6 m	95%	Partial Cloud	18	0-15
Second Spray								
Day 0	19.7	July 21	Spruce	3-8 m	40%	Overcast	12	20
Day 0	20.4	July 21	Spruce	10 m	40%	Overcast	1	20
Day 0	10.8	July 21	Fir	2-3 m	70%	Overcast	8	Light
Day 0	16.0	July 21	-	3 m	40%	Overcast	10	Light
Day 0	22.0	July 21	-	2 m	25%	Overcast	10	Light
Day 0	16.0	July 21	Fir	2 m	30%	Overcast	8	Light
Day 0	25.3	July 21	-	4-5 m	90%	Overcast	10	Light
Day 1	22.8	July 22	Fir & Birch	2-3 m	90%	Clear	15	20
Day 1	24.9	July 22	Fir & Birch	4-5 m	80%	Clear	17	20
Day 1	18.9	July 22	Fir	2-3 m	80%	Clear	18	20
Day 1	20.8	July 22	Fir	3-5 m	60%	Sunny	18	10-20
Day 1	23.6	July 22	Fir & Birch	3 m	60%	Clear	19	20
Day 1	11.2	July 22	Spruce	1-4 m	40%	Sunny	20	20
Day 1	7.4	July 22	Spruce	1-4 m	40%	Sunny	20	20
Day 1	16.7	July 22	Fir	3-5 m	20%	Sunny	20	20
Day 3	18.9	July 24	Fir	2-3 m	70%	Overcast	17	Light
Day 3	17.0	July 24	Fir	3-6 m	80%	Sunny	20	0-5
Day 3	20.4	July 24	Fir	2 m	20%	Sunny	20	0-5
Day 3	19.7	July 24	Spruce	1-3 m	100%	Overcast	13	0-10
Day 3	19.7	July 24	Fir	10 m	80%	Overcast	13	0-5
Day 3	20.8	July 24	Fir	3-5 m	40%	Overcast	13	0-5
Day 3	11.2	July 24	Spruce	5-8 m	80%	Overcast	13	0-5
Day 5	22.8	July 26	Fir	3 m	20%	Clear	22	15
Day 5	20.8	July 26	Fir	3-6 m	60%	Clear	18	Light
Day 5	20.4	July 26	-	6 m	60%	Clear	22	Light
Day 5	14.5	July 26	-	3 m	40%	Clear	22	Light
Day 5	20.0	July 26	-	1 m	10%	Clear	20	0-5
Day 5	11.2	July 26	Spruce	7-9 m	80%	Partial Cloud	24	0-10
Day 7	23.6	July 28	Fir	4 m	70%	Overcast & Rain	12	0-5
Day 7	18.9	July 28	Birch & Fir	2 m	60%	Rain	12	0-5
Day 7	17.4	July 28	Birch & Fir	2-3 m	60%	Overcast	12	0-5
Day 7	22.8	July 28	Spruce	5-7 m	50%	Overcast	12	0-5
Day 7	18.9	July 28	Birch & Fir	3 m	50%	Overcast	12	0-5
Day 7	20.0	July 28	Birch & Fir	2-3 m	80%	Overcast	12	0-5
Day 7	21.2	July 28	Birch	4 m	60%	Overcast	12	0-5
Day 7	18.5	July 28	-	3 m	30%	Overcast	14	Light
Day 7	17.4	July 28	-	4 m	50%	Overcast	14	Light
Day 7	24.1	July 28	-	7 m	75%	Overcast	14	Light
Day 7	24.5	July 28	-	3 m	85%	Overcast	14	Light

[1] Fir - *Abies balsamea*
Birch - *Betula papyrifera*
Tamarack - *Larix laricina*
Willow - *Salix* sp.
Spruce - *Picea* sp.
Alders - *Alnus* sp.
Maple - *Acer* sp.

[2] Light - 0-5
Moderate - 5-10
Strong - >10

SPRAY BLOCK 115

Figure C5: Distribution of White-throated Sparrow collection in Block 115

Table C5 : White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Figure C.8

DISTRIBUTION OF WHITE-THROATED SPARROW COLLECTION IN BLOCK 118

LEGEND

First Spray	Second Spray
1 Day of spray	4 Day of Spray
2 Day after spray	5 Day after spray
3 Third day after spray	6 Third day after spray
	7 Fifth day after spray
	8 Seventh day after spray

——— Spray line

····· Shut off

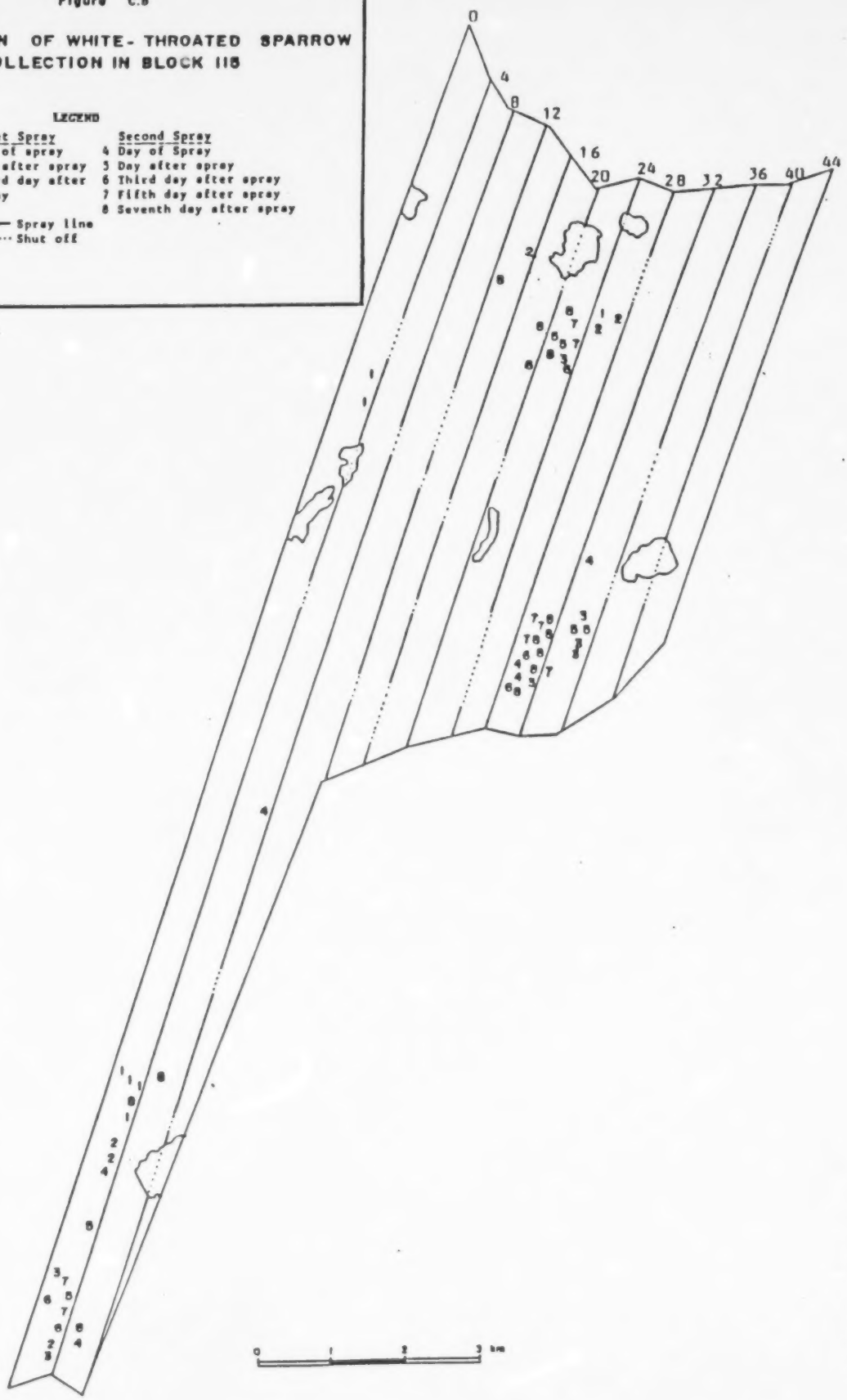

Table C3: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

BLOCK 113

Collection Day	ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather: Sky	Weather: Temp. (°C)	Weather: Wind (km/hr)[2]
First Spray								
Day 0	23.2	July 7	Spruce	5 m	40%	Clear	20	Light
Day 0	29.8	July 7	Spruce	3 m	70%	Clear	20	Light
Day 0	20.8	July 7	Fir	2-3 m	70%	Clear	20	5
Day 0	17.4	July 7	Fir & Deciduous	3 m	85%	Clear	20	Light
Day 0	16.0	July 7	-	5-7 m	80%	5% Cloud	20	5
Day 0	22.8	July 7	-	2-3 m	95%	Clear	20	5-10
Day 0	29.8	July 7	-	2 m	-	Partial Cloud	15	10
Day 1	17.8	July 8	-	-	0%	Rain	10	0-5
Day 1	18.5	July 8	Fir	5-8 m	100%	Rain	10-15	0-5
Day 1	6.1	July 8	-	-	0%	Overcast	10	0-5
Day 1	13.8	July 8	Spruce	1-2 m	50%	Overcast	15	0-5
Day 1	12.2	July 8	-	1-3 m	30%	Overcast	10-15	0-10
Day 1	16.7	July 8	Birch	5-6 m	35%	Overcast	15	5
Day 3	23.6	July 10	-	7-8 m	5%	Overcast	10	Light
Day 3	16.7	July 10	Fir	7 m	90%	Overcast	10	Light
Day 3	23.6	July 10	Fir	7 m	30%	Overcast	12	Light
Day 3	18.5	July 10	Fir	1 m	5%	-	-	-
Day 3	20.4	July 10	Fir	9 m	100%	90% Cloud	12	5
Day 3	18.1	July 10	-	3-10 m	5%	Fog	10	0-10
Day 3	17.4	July 10	-	3-10 m	10%	Clear	18	5
Second Spray								
Day 0	22.8	July 18	-	7 m	60%	Clear	18	Light
Day 0	18.1	July 18	Fir	2-3 m	5%	5% Cloud	18	5-10
Day 0	24.1	July 18	-	4 m	65%	Clear	22	Strong
Day 0	18.1	July 18	-	6 m	80%	Clear	22	Strong
Day 0	9.9	July 18	-	2 m	10%	Clear	22	Strong
Day 0	26.2	July 18	Spruce & Fir	5-6 m	40%	Clear	22	20
Day 1	17.4	July 19	-	7-8 m	50%	Overcast & Fog	12	0-5
Day 1	19.7	July 19	Maple	5-8 m	50%	Overcast & Fog	12	0-5
Day 1	24.5	July 19	-	7-8 m	50%	Overcast	12	0-5
Day 1	29.8	July 19	-	5-8 m	50%	Overcast	12	0-5
Day 1	13.2	July 19	-	-	0%	Partial Cloud	15	0-5
Day 1	16.0	July 19	-	-	0%	Partial Cloud	15	0-5
Day 3	20.0	July 21	Fir & Birch	2-3 m	60%	Overcast	14	20
Day 3	22.8	July 21	-	7 m	60%	Overcast	10	Moderate
Day 3	18.9	July 21	Fir	2 m	10%	Overcast	10	Moderate
Day 3	17.0	July 21	-	8 m	80%	Overcast	10	Moderate
Day 3	18.9	July 21	-	3 m	50%	Overcast & Rain	8	5-10
Day 3	18.5	July 21	-	5-8 m	50%	Overcast & Rain	8	5-10
Day 3	17.4	July 21	Fir	5-8 m	50%	Overcast & Light Rain	8	10
Day 5	16.0	July 23	Fir	1-2 m	50%	Overcast	10	Light
Day 5	13.2	July 23	Fir	1-3 m	25%	Overcast	10	Light
Day 5	18.9	July 23	-	-	-	Overcast	10	Light
Day 5	12.5	July 23	-	-	-	Overcast	10	Light
Day 5	21.2	July 23	Spruce	8 m	40%	Overcast	12	0-10
Day 5	20.4	July 23	Fir	2 m	5%	Overcast	12	0-10
Day 5	28.9	July 23	Birch	2 m	50%	70% Cloud	20	Light
Day 5	22.0	July 23	Fir	7-8 m	40%	Partial Cloud	15	10
Day 7	16.7	July 25	Spruce	3-6 m	50%	Overcast	17	Light
Day 7	22.0	July 25	-	4 m	50%	Overcast	14	Light
Day 7	18.5	July 25	-	6 m	15%	Overcast	14	Light
Day 7	13.2	July 25	-	1-2 m	5%	Overcast	14	Light
Day 7	20.4	July 25	-	1 m	5%	Overcast	13	Light
Day 7	20.0	July 25	Fir	1-2 m	20%	Overcast	14	Light
Day 7	21.2	July 25	Maple	2-3 m	5%	Overcast	12	5-10
Day 7	17.4	July 25	Spruce	5 m	60%	Partial Cloud	14	0-10
Day 7	20.8	July 25	Fir & Spruce	10 m	100%	Partial Cloud	14	0-10
Day 7	18.9	July 25	Maple	2-3 m	30%	Overcast	14	0-10
Day 7	21.2	July 25	Spruce	3-5 m	30%	Partial Cloud	18	5-10

1 Fir - *Abies balsamea*
Birch - *Betula papyrifera*
Tamarack - *Larix laricina*
Willow - *Salix* sp.
Spruce - *Picea* sp.
Alders - *Alnus* sp.
Maple - *Acer* sp.

2 Light - 0-5
Moderate - 5-10
Strong - >10

CONTROL

Table C6: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collection

Table C6: White-throated Sparrow ChE activity, forest cover and relevant meteorologic conditions at time of collect

CONTROL

ChE Value	Collection Date	Forest Cover[1]	Tree Height	Percent of Canopy Cover	Weather: Sky	Temp. (°C)	Wind (km/hr)[2]
26.2	June 28	-	5-8 m	100%	Overcast	14	0-5
24.5	June 28	-	5-8 m	50%	Overcast	14	0-5
28.9	June 29	Scrub	2-3 m	50%	Partial Cloud	19	0-5
20.4	June 29	-	5-8 m	70%	Partial Cloud	15	0-5
24.1	July 1	Old Cutover	3 m	30%	Partial Cloud	14	0-5
26.6	July 1	-	3 m	30%	Partial Cloud	14	0-5
26.6	July 1	Scrub	-	0%	Partial Cloud	14	0-5
24.1	July 1	Scrub	-	50%	Partial Cloud	14	10
23.6	July 1	-	1-2 m	90%	Partial Cloud	14	10
23.6	July 1	Fir	3 m	-	Overcast	10	Light
23.6	July 1	-	3 m	-	Overcast	10	Light
27.1	July 1	-	3 m	-	Overcast	10	Light
27.1	July 1	-	3 m	-	Overcast	10	Light
26.2	July 2	Mixed	1-3 m	80%	Overcast	10	Light
28.9	July 2	Fir	1-2 m	70%	Heavy Rain	10	Light
28.9	July 2	Spruce & Fir	3 m	40%	Rain	10	Light
25.3	July 3	-	-	0%	Partial Overcast	15	Moderate
28.4	July 3	-	1-3 m	40%	Partial Cloud	18	Moderate
30.7	July 3	-	1-2 m	30%	Clear	18	Moderate
25.3	July 18	Birch	4-5 m	90%	Clear	15	10

1 Fir - Abies balsamea
Birch - Betula papyrifera
Tamarack - Larix laricina
Willow - Salix sp.
Spruce - Picea sp.
Alders - Alnus sp.
Maple - Acer sp.

2 Light - 0-5
Moderate - 5-10
Strong - >10

APPENDIX D

Table D1: Details of sample collection data

Table D2: Number and percentage of birds with ChE activity below critical value (20.8 units) and life-threatening value (13.0 units) for all blocks

Table D1: Details of sample collection data

Block Number		SPRAY 1 Collection Day 0	1	3	SPRAY 2 Collection Day 0	1	3	5	7
108A	n	6	5	6	6	6	10		
	$\bar{x}$	19.8	17.3	18.4	21.4	15.7	16.2		
	SD	4.9	6.2	5.1	3.8	3.8	3.2		
	range	14.5-28.0	6.8-22.8	12.5-24.5	16.7-25.3	11.8-20.4	9.9-21.2		
108B	n	7	7	6					
	$\bar{x}$	20.6	21.6	20.9					
	SD	5.7	4.9	4.4					
	range	11.8-27.5	14.5-26.2	14.5-25.7					
109	n	7	7	7	6	7	9	8	9
	$\bar{x}$	24.7	16.0	20.6	22.5	19.8	19.3	19.2	22.5
	SD	2.8	5.2	4.5	3.4	4.1	5.1	4.0	4.4
	range	19.7-26.6	9.2-23.6	13.8-27.1	18.1-26.6	15.2-22.8	10.5-24.5	13.2-24.5	16.7-28.4
113	n	6	6	6	7	8	7	6	11
	$\bar{x}$	21.9	17.8	21.3	18.6	18.3	18.2	18.3	20.7
	SD	3.3	5.1	4.3	4.7	6.2	3.3	4.4	7.3
	range	10.8-25.3	9.9-22.0	14.5-26.6	10.8-25.3	7.4-24.9	11.2-20.8	11.2-20.8	17.4-24.5
115	n	7	6	7	6	6	7	8	11
	$\bar{x}$	22.8	14.2	19.8	19.9	20.1	19.1	19.1	19.1
	SD	5.4	4.6	2.9	5.9	6.1	1.9	5.3	2.6
	range	16.0-29.8	6.1-18.5	16.7-23.6	9.9-26.2	13.2-29.8	17.4-18.9	12.5-28.9	13.2-22.2
Control	n	20							
	$\bar{x}$	26.0							
	SD	2.5							
	range	20.4-30.7							

Table D2: Number and percentage of birds with ChE activity below critical value (20.8 units) and life-threatening value (13.0 units) for all blocks

Spray	Block	Day	n	Number Below 20.8	%	Number Below 13.0	%
1	108A	0	6	4	67	0	-
		1	5	3	60	1	20
		3	6	4	67	1	17
2	108A	0	6	3	50	0	-
		1	6	6	100	3	50
		3	10	9	90	2	20
1	108B	0	7	4	57	1	14
		1	7	3	43	0	-
		3	6	3	50	0	-
1	109	0	7	1	14	0	-
		1	7	5	71	2	29
		3	7	3	43	0	-
2	109	0	6	2	33	0	-
		1	7	4	57	0	-
		3	9	5	56	2	22
		5	8	5	62	0	-
		7	8	4	50	0	-
1	113	0	6	1	17	0	-
		1	6	3	50	1	17
		3	6	3	50	0	-
2	113	0	7	5	71	1	14
		1	8	3	37	2	25
		3	7	6	86	1	14
		5	6	4	67	1	17
		7	11	6	54	0	-
1	115	0	7	2	29	0	-
		1	6	6	100	2	33
		3	7	5	71	0	-
2	115	0	6	3	50	1	14
		1	6	4	67	0	-
		3	7	6	86	0	-
		5	8	5	62	1	20
		7	11	7	64	0	-
Totals and % for all blocks combined			233	137	58.8%	22	9.4%

APPENDIX E

Table E1: Summary results of 1-tailed T-tests comparing mean of each day to critical value ($H_0: \bar{X} \geq 20.8$ $H_A: \bar{X} < 20.8$)

Table E1: Summary results of 1-tailed T-tests comparing mean of each day to critical value ($H_0: \bar{x} \geq 20.8$ $H_A: u < 20.8$) i.e. is the ChE activity on each day significantly less than the critical value of 20.87 (NS - not significantly different
S - significantly different)

Spray 1 (all blocks combined)			Spray 2				
0	1	3	0	1	3	5	7
			With 108A				
t=1.53 d.f.=32 p>0.50 NS	t= -3.37 d.f.=30 p<0.001 S	t= -0.77 d.f.=31 p>0.50 NS	t= -0.32 d.f.=24 p>0.50 NS	t=2.29 d.f.=26 p<0.01 S	t=4.16 d.f.=32 p<0.0005 S	t=1.99 d.f.=21 p<0.025 S	t=0.27 d.f.=30 p>0.50 NS
			Without 108A				
			t=0.52 d.f.=18 p>0.50 NS	t=1.28 d.f.=20 p>0.050 NS	t=2.44 d.f.=22 p<0.01 S	t=1.99 d.f.=21 p<0.025 S	t=0.27 d.f.=30 p>0.05 NS
			108A Alone				
			NS	S	S		